THE JOURNAL OF NUTRITION

OFFICIAL ORGAN OF THE AMERICAN INSTITUTE OF NUTRITION

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VOLUME 60

SEPTEMBER – DECEMBER 1956

WITH

SUPPLEMENT No. 1, OCTOBER

SUPPLEMENT No. 2, NOVEMBER

PUBLISHED MONTHLY BY

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA, PENNSYLVANIA

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ERRATUM

Vol. 60, page 151, starting with middle of line 6 of section 9, should read as follows:

Approval of the committee report that C. G. King serve as President of the 1960 International Nutrition Congress and that C. G. King serve as American Institute of Nutrition delegate to the 1957 International Nutrition Congress in Paris, France.

LAFAYETTE BENEDICT MENDEL

(1872 – 1935)

LAFAYETTE BENEDICT MENDEL

LAFAYETTE BENEDICT MENDEL

(February 12, 1872 – December 9, 1935)

On the Sheffield campus at Yale stands a brown stucco mansion, soon to be removed for the extension of the Electrical Engineering Building. This house is fondly remembered by a diminishing number of American biochemists as the laboratory in which Professor Lafayette B. Mendel held his classes and conducted research for almost a third of a century. The office room was restricted; the necessity for employing irregular spaces such as the original parlor and the art gallery, led to make-shift adjustments; and the laboratory equipment, even by the standards of those simpler days, was inadequate. Yet from this laboratory issued not only the record of fundamental research of a high order of excellence, but also several generations of investigators and teachers, most of whom later occupied chairs of biochemistry in widely scattered academic centers in our country. Although in 1923, the Department of Physiological Chemistry was moved to more adequate quarters in the Medical School, the accomplishments in those early years alone are proof of the scientific acumen, the pedagogical genius, and the administrative skill of Doctor Mendel.

The education of Mendel was rather typical of his time; after graduating from Yale in 1891 (he was then 19), he set out to prepare himself for a scientific career. His outlook was profoundly influenced by Chittenden who had established at Yale the first teaching laboratory in physiological chemistry in this country. Mendel became Chittenden's assistant and carried on graduate work which led to the Ph.D. degree in 1893. He remained at Yale as an instructor in the Sheffield Scientific School for two years before deciding to avail himself of the instruction and research opportunities afforded

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by European laboratories. He was well equipped intellectually and had become a proficient chemist. He went to Breslau where he studied first with Heidenhain and later with Röhmann. Despite the fact that Mendel was prepared to personally provide material as well as apparatus, permission to work in Heidenhain's laboratory was not given until the new American student exhibited what was to Heidenhain a novelty — a sample of crystallized protein. From the experience in Breslau began Mendel's interest in experimental physiology and the technical approach to it; he always felt that Heidenhain had a great influence upon his subsequent scientific activities. From Breslau Mendel went to Freiburg where for a time he studied chemistry with Baumann.

Upon returning in 1896, Mendel was appointed Assistant Professor in Chittenden's laboratory at Yale. Here began a period of teaching and extraordinary research activity which was to continue for almost 40 years. The early investigations reflect somewhat the interest of his mentor in the chemistry of digestion and absorption, although as early as 1898, in a paper on the nutritive value of fungi, he showed his developing interest in the new science of nutrition. Until about 1910, however, Mendel continued to look into the many paths in the area of gastrointestinal physiology, the beginning of wisdom for anyone interested in nutritional biochemistry. With increasing independence and with the help of numerous graduate students, he examined the enzymatic factors in digestion and the avenues of absorption of various nutrients. An extensive study of the pathway of excretion of certain inorganic salts was made as well as of the intermediary metabolism of purines. In 1905 and 1906 a series of papers on the physiology of the molluscs was published; this activity shows the catholicity of his interest in comparative physiological chemistry upon which he drew so effectively for his delightful lectures. During the following two years (1907 to 1908) several papers appeared dealing with various phases of the chemical physiology of the embryo. Toward the end of this period, studies on the absorption of fat and on the utilization of carbohydrate

and protein were carried out and a long series of experiments on creatine and creatinine was reported. It is of interest that about this time occasional papers were published in the German journals.

Mendel's growing interest in nutrition and his familiarity with the then current methods of experimentation in this field, led him to the conclusion that only through the use of purified experimental diets could definitive information on the nutritive significance of foodstuffs, notably the proteins, be secured. He felt that he needed the assistance of a chemist skilled in the field of protein chemistry for the most effective prosecution of this program of investigation. What were the details of the initial meeting of minds of Osborne and Mendel is unimportant here; it is important that in New Haven, there were these two scholarly men, each in a situation which permitted him to engage in a scientific collaboration under almost ideal conditions, a circumstance to prove extraordinarily fortunate for the progress of science in America. It would not be easy to find two more diverse personalities: Osborne, the shy, retiring savant, and Mendel, the sociable, extrovert scholar with unusually broad interests. However, they possessed one attribute in common, namely, a deep personal and professional regard for the attainments and intellectual honesty of the other. During the almost 20 years of the collaboration, each learned much from the other and each exerted a searching critical influence upon the planning of the experiments as well as on the preparation of the papers, most of which are models of expository writing.

When Osborne and Mendel began their work, one of the most discussed questions in the field of nutrition was the optimal quantity of protein in the diet. Liebig, Moleschott, Voit, Rubner, Hindhede, Chittenden and McCay, among others, had discussed the problems from various points of view with various emphases, and with little unanimity of conclusion. Osborne and Mendel initially set for themselves the task of examining the importance of the quality of dietary protein

in nutritive success or failure, a decision which naturally turned the searchlight of their inquiry upon the amino acids. Aided by a continuing grant from the Carnegie Institution of Washington, the research program was begun at the Connecticut Agricultural Experiment Station in New Haven; at the outset the details of the breeding, housing and feeding of the albino rat were worked out. The observations arising from these important preliminary experiments were published in a monograph in 1911. The addition of this broad research activity to an already heavy program of investigation, teaching and general university and public service, illustrates the astounding capacity for work shown by Mendel at this stage of his career.

Once Osborne and Mendel had established the experimental procedure, a broad plan of study was initiated. The unusually capable staff in Osborne's laboratory contributed to the preparation of purified proteins from various sources as well as to the analytical values for their content of the various amino acids. Papers by Osborne and Mendel began to appear in considerable numbers — 6 in 1912, 10 in 1915–16, 14 in 1917–18, and so on at an average rate of some 8 papers a year between 1911 and 1927! There were studies on the determination of the comparative nutritive value of various purified proteins from cereal grains, other seeds and plant tissues, for growth and maintenance. This inevitably led to the supplementation of some of the deficient proteins with the missing amino acids and to the basic concept that some of the amino acids derived from the hydrolysis of proteins cannot be readily synthesized by the animal body but must be provided *de novo* in the diet. Within the limits of the experimental procedure, protein minima for growth and maintenance were determined and the minimum daily requirement of certain amino acids was suggested.

Following the initial work on purified proteins, each item in the simplified experimental ration was scrutinized. It was in connection with the examination of the needs of the rat

for inorganic salts that Osborne and Mendel became convinced that in natural foods there were present nutritionally indispensable factors for whose detection and quantitative estimation, methods were then non-existent. Thus, as a result of their demonstration that a mixture of pure salts similar to those found in milk, failed to support nutritive success as did their "protein-free milk" used to provide the ash constituents in their early diets, they were receptive to the suggestion that an indispensable water-soluble factor (vitamin B) was present in milk. Later, a study of the lipid component of the diet, brought out the fact that certain natural fats possess nutritive virtues which are lacking in others, an observation marking their discovery of vitamin A. The natural distribution of this factor and its chemical character and behavior also received attention.

In the course of the evaluation of the carbohydrate portion of the diet, Osborne and Mendel experimented with diets, the components of which were present in extreme proportions. One of the by-products of this line of research was their interest in the response of the kidney to diets unusually rich in protein. The hypertrophic response of the kidney, however, was but one aspect of the larger question of growth which early elicited their interest. Whereas the previous concept of growth was that of an inherited capacity which must act at the proper time (youth) to exert its influence, Osborne and Mendel showed that growth can occur at any time in the life of an animal, once the chemical environment (nutrition) is adequate, other factors being optimal.

The last contribution published by Osborne and Mendel was a summary of their work on growth; after a fruitful period of some 20 years, this scientific partnership came to an end with the death of Osborne in 1929. The work of Osborne and Mendel gave direction to the development of nutritional biochemistry in this country: subsequent extension of the field of essential amino acids, discoveries based on the use of fat-free diets and the extended differentiation between the vitamins, are expansions of our knowledge of nutrition

whose point of departure is the use of experimental rations composed of known purified components.

As stated before, Mendel supervised investigations on a wide variety of topics in chemical physiology in his own laboratory at Yale during and after his work with Osborne at the Experiment Station. The metabolism of calcium and magnesium, the physiology of absorption, transport and secretion, the metabolism of the pyrimidines, non-specific protein reactions, the regulation of blood volume, carbohydrate metabolism, the physiology and distribution of the vitamins, are some of the topics to which he added significant knowledge through collaboration with graduate students. The relation of the chemical character of dietary fat to that of body fat was one of the themes which appealed especially to Professor Mendel and he gave it his enthusiastic attention.

Mendel was a prolific writer; along with his diverse program of investigation he wrote many reviews and editorials on the relation of the growing science of nutrition to medicine, and on the influence of nutritional research in the national economy. Although there are some 340 papers attributed to him, this number is by no means an index of the number of investigations which he directed, for with characteristic generosity, the inclusion of the "Professor's" name on a paper arising from collaboration with a student was left to the choice of the junior partner. Doctor Mendel published three books: "Childhood and Growth," (1906), "Changes in the Food Supply and their Relation to Nutrition," (1916), and "Nutrition, the Chemistry of Life," (1923). He wrote fluently in an accurate and cogent manner and the first draft of his manuscripts rarely required revision.

Mendel's laboratory was an active center of both undergraduate and graduate instruction and he is affectionately remembered by the large number of his students as an inspiring teacher. Early in his career his students were largely undergraduates; in many of these he aroused interest in the new field of physiological chemistry and led them to realize the opportunities in medicine, bacteriology, public health and

physiological chemistry itself, stimulating them to undertake advanced study in these fields. Mendel's outlook was pragmatic and in this attitude lay his strong appeal to young men. He believed that the investigator should be able to exploit his own work effectively and to this end he required that the answer to a classroom question or the more extended seminar report be given not only accurately, but also, as he so often said, in elegant English. He himself was an easy but forceful speaker and ranged widely for illustrations for his argument. His demonstrations were carefully planned and executed with finesse, always accompanied by lucid explanation; and when the inevitable uncertainty of animal experimentation raised its ugly head, the situation was usually saved by an anecdote from his past experience or by bits of delicate humor. He strove to inculcate a respect for skilled laboratory technique; he permitted no liberties with accuracy or neatness on the part of the student. A confirmed experimentalist, he was extremely sensitive to lack of care in handling animals and insisted that adequate anesthesia and gentle manipulation be routine in the demonstration or experiment.

Graduate students began to come to Mendel early in his career; his extraordinarily broad knowledge of the literature in physiology and biological chemistry enabled him to suggest a great range of topics worthy of study. Although infrequently assigning a student to a detailed study of an analytical method, Mendel was not without a real appreciation of methodology, for he frequently said "Give me a method and I will give you a problem." His main interest, however, was function, approached by the physiological and chemical methods then current and this dynamic point of view was a factor in attracting graduate students. The beginner was literally guided through the intricacies of technique by the "Professor's" own hand; as time went on he became aware of a growing maturity in his own scientific philosophy under the deft guidance of his advisor. Mendel not only schooled his men in the tenets of research, but in many instances was

able to endow them with a benign but liberal independence in scientific thinking. Graham Lusk wrote of Mendel as a teacher:

> "He has been the guide, philosopher and friend to many young men and women; he has encouraged them to walk by themselves when they were able to stand alone; and he has given wise counsel in times of difficulty. Herein he has shown himself as one of the great teachers of his time."

Probably no part of Professor Mendel's teaching program will be more pleasantly recalled by the participants than his seminar. These stimulating weekly sessions of informal discussion by both the "Professor" and the students, still seem to the writer to be an unequalled teaching device for the advanced student with respect to contact with the problems and the progress in as broad a field as is physiological chemistry.

Richly endowed with factual knowledge, with an engaging personality, and with a deep interest in his students, he gave serious consideration to the methods of pedagogy and to the dignity of the profession of teaching. The prepared mind and a willingness to work hard were for him evidence enough that the student merited his guidance. He almost literally looked upon his graduate students as his "laboratory family" and took great satisfaction in the distinguished subsequent careers of many of them.

Mendel's outstanding position in American science and his gift for administration early led the University to make use of his talents. A member of the faculty for 43 years, he served variously on the governing boards of the Sheffield Scientific School, the Graduate School, the University Library Committee, and the Board of Permanent Officers of the Medical School. A keen judge of men, it was natural that he should have served at various times on fellowship committees and on the Admissions Board of the Undergraduate Schools. Appreciation of Mendel's University service is shown by his appointment in 1921 to one of the first Sterling Professorships.

Outside the University, Mendel's advice and assistance was sought in the fields of nutrition, public health, and medicine. He was an official member of several international congresses. He recognized the importance of organized effort of scientific groups; thus he served as secretary of the American Physiological Society for several of its early years and was active in the formation of the American Society of Biological Chemists in 1906, serving this group in the various offices. He was the first President of the American Institute of Nutrition and helped guide the Editorial policy of the Journal in its early years. He enjoyed scientific editorial work and performed important service of this nature for *Chemical Abstracts,* the *Journal of Biological Chemistry, Scientific Monographs* of the American Chemical Society, and the *Journal of the American Medical Association.* In view of the meticulous attention given by him to these extra duties, one marvels again at Mendel's enormous capacity for work.

Doctor Mendel's career spanned the period of the development of nutrition and he was looked upon as one of the leading protagonists of this new area in the field of physiological chemistry. His prolific contributions to the scientific literature and his effectiveness as a speaker, led to many such engagements. In 1906 he gave one of the first Harvey Lectures and again in 1914 he discussed the chemical aspects of growth before that society. He spoke on one of the Sigma Xi lecture tours and gave the 1914 Herter Lectures at University and Bellevue Hospital Medical College in New York. Nine years later he gave the Hitchcock Lectures at the University of California. In 1930 he lectured on the Schiff Foundation at Cornell University and was Cutter Lecturer on Preventive Medicine at the Harvard Medical School. During all of this time he was in demand as a speaker to civic and scientific groups; indeed, he can be looked upon as one of the early outstanding forces in the popularization of science.

It seems inevitable that Dr. Mendel's broad interest in physiology, toxicology and chemistry should have brought him into more or less close contact with medicine. For the

greater part of his professional life, he advised on national medical problems, wrote for medical journals, and spoke to medical audiences. As he took part in the development of nutrition he became convinced, and so urged, that it be looked upon as an exceedingly important factor in preventive medicine. He was frequently consulted by the practitioner regarding clinical problems; the discussion usually revolved about normal physiology and chemistry upon the basis of which guidance to the solution of the problem was usually given.

During his lifetime many honors came to Dr. Mendel. He was proud to have been a charter member of the Yale Chapter of Sigma Xi. Later, honorary degrees were conferred by the University of Michigan, Rutgers University, and Western Reserve University. He was long a member of the National Academy of Sciences and of the American Philosophical Society. In 1929 he was elected to membership in the Societe de Biologie in Paris and became a member of the American Academy of Arts and Sciences a year later. His academic service was recognized by a gold medal given by the American Institute of Chemists and a year before his death, the Chemists' Club of New York conferred upon him the Conne Medal for outstanding chemical service to medicine. When he was 60, his friends, students, and professional associates presented him with his portrait and for the same occasion, there was published an Anniversary Number of the *Yale Journal of Biology and Medicine* containing articles by some of his former pupils. While he valued these various honors, he prized most of all the successes of his many former students.

In Dr. Mendel's death on December 9, 1935, at the age of 63, there passed not only one of the pioneers in the science of nutrition, but also a gentle friend to many whose lives were enriched by contact with him.

ARTHUR H. SMITH
Wayne State University

STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT TREATMENT [1]

IV. ETHYL ESTERS OF HEAT-POLYMERIZED LINSEED, SOYBEAN AND SUNFLOWER SEED OILS

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(Received for publication March 31, 1956)

INTRODUCTION

The literature on the effects of polymerization temperature on the nutritive value of edible oils has been reviewed up until 1952 by Crampton et al. ('53). Since then Frahm et al. ('53) have reported deleterious effects of heat-polymerized whale oil when it is fed to mice. Raju and Ragagopalan ('55) have reported the results of feeding rats with diets containing 15% of peanut or sesame or cocoanut oil which had been heated at 270°C. in open pans in contact with air. The effects included depression of live weight gain, decrease in food efficiency and increases in liver weight as percentage of body weight and in percentage of liver fat. However, although the temperature used by Raju and Ragagopalan suggests that there was polymerization, the experiments of these workers are not comparable with those carried out in our laboratories where the oils were heated in a current of CO_2. Kaunitz et al. ('55) have found that cottonseed oil heated and aerated at 90 to

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Province of Quebec, Canada. Journal Series no. 394.

95°C. for periods of up to 300 hours became injurious to rats. Incorporation of fresh oil gave a degree of protection against some of the deleterious effects; peroxides were considered not likely to be responsible for the ill effects. The conditions of heating differed greatly from those used in our laboratories.

In a previous paper (Crampton et al., '53) we have reported a study of the nutritional properties of certain fractions prepared from the ethyl esters of heat-polymerized linseed oil by distillation and urea adduct formation. The preparation and the designations of the different fractions are described in this previous paper, as are also the diets and plan of the feeding trials. For convenience of reference the flow sheet for fractionation is reproduced in figure 1.

It should be pointed out here that polymerization of triglyceride oils is now known definitely to include formation of significant amounts of trimeric, and even of some higher polymeric acyl radicals, as well as of dimeric acyl radicals (Paschke and Wheeler, '54). In the present paper, therefore, fraction 6 is designated "polymer" rather than "dimer."

From this earlier work it seemed reasonable to suppose that heated linseed oil was nutritionally injurious, firstly, because of the presence of polymerized material that is poorly absorbed, if at all, and secondly, because of the presence of monomeric acyl radicals incapable of forming urea adducts by reason of some structural feature, possibly a cyclization. In this connection it is noteworthy that Paschke and Wheeler ('55) have now demonstrated the formation of a cyclic monomer during heat polymerization of methyl eleostearate and have shown that this cyclic monomer is mainly an orthodisubstituted cyclohexadiene.

The question that next presented itself was the degree to which formation of non-adduct-forming monomeric material could be related to the fatty acid composition of the original oil itself. Accordingly, in 1953-'54 further rat feeding trials were carried out involving 24 lots of 10 animals each in order to study the nutritional value of similar fractions prepared from soybean oil and sunflower seed oil. Soybean oil was

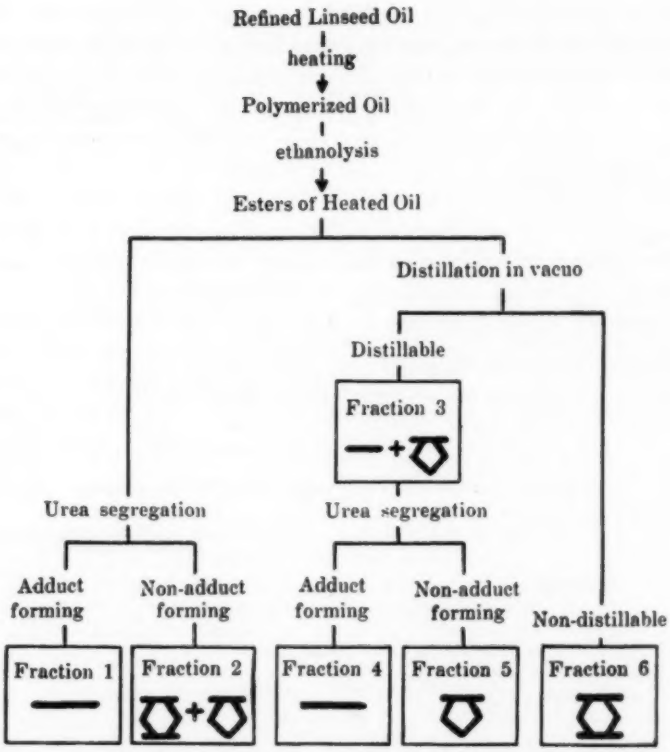


Fig. 1 Flow sheet illustrating preparation of fractions of esters of heated linseed oil used in feeding trials.

chosen because it is a food oil that contains some linolenic acid. Sunflower seed oil was chosen because of its high linoleic acid content and negligible linolenic acid content. Typical data for the fatty acid composition of the three oils are summarized in table 1.

Preliminary experiments with soybean and sunflower seed oils showed that non-adduct-forming distillable ester (NAFD) fractions could be obtained from both oils using the same polymerization temperature (275°C.) and heating in a current of CO_2. It was necessary to heat these oils for longer times

TABLE 1

Fatty acid composition by weight of linseed, soybean and sunflower seed oils

	SATURATED			UNSATURATED		
	C_{16}	C_{18}	C_{20}	Oleic	Linoleic	Linolenic
	%	%	%	%	%	%
Linseed N. American (I No. 179.8)[1]	6.3	2.5	0.5	19.0	24.1	47.4
Soybean (I No. 132.5)[2]	10.6	2.4	2.4	23.5	51.2	8.5
Sunflower seed[3]	6.4	1.3	4.0	21.3	66.2	..

[1] Rose and Jamieson ('41), cited by Bailey ('52).

[2] Hilditch, Meara and Holmberg ('47), cited by Bailey ('52).

[3] Barker, Crossley and Hilditch ('50), cited by Bailey ('52).

TABLE 2

Yields of fractions of ethyl esters used in feeding trials

FRACTION	YIELD AS PER CENT OF TOTAL ETHYL ESTERS OF HEATED OIL		
	Linseed[1] (12 hr. at 275°C.)	Soybean (24 hr. at 275°C.)	Sunflower seed (26 hr. at 275°C.)
1. Adduct-forming fraction of total esters	46 (293)[2]	63 (296)	65 (..)
2. Non-adduct-forming fraction of total esters	54 (472)	37 (415)	35 (..)
3. "Distillable" esters	60 (294)	74 (296)	75 (303)
4. Adduct-forming fraction of distillable esters	49 (293)	64 (294)	65 (295)
5. Non-adduct-forming fraction of distillable esters	11 (300)	10 (294)	10 (296)
6. Non-distillable esters	40 (550)	26 (415)	25 (613)

[1] Data for linseed oil quoted from Crampton et al. ('53).

[2] Figures in parentheses are cryoscopic mean molecular weights.

than linseed oil in order to attain reasonable yields of this fraction. The times used for bulk preparation of fractions are given in table 2, together with approximate yields of the various fractions. The data for linseed oil are quoted from Crampton et al. ('53).

RESULTS

Tables 3 to 7 summarize the results of the feeding experiments with soybean oil and sunflower seed oil, the data for linseed oil being cited from Crampton et al. ('53). In the discussion that follows, the data for linseed oil are considered along with those for the two other oils; the latter sets of data are here reported for the first time.

Survival of rats. The percentages of the rats surviving the 28-day feeding are shown in table 3.

TABLE 3

Comparison of the nutritional effects of ethyl esters of linseed, soybean and sunflower seed oils

(Ten rats per lot)

Percentage of rats surviving 28-day test

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
1	——	100	100	100	100	100	100
2	⟨⟩ + ◇	20	100	0 [1]	100	50 [2]	50 [2]
3	—— + ◇	90	100	100	100	100	100
4	——	100	100	100	100	100	100
5	◇	0	0	100	80 [3]	100	100
6	⟨⟩	70	100	0 [1, 4]	10 [4]	100	100

[1] All animals removed after 10 days because of diarrhea and extreme viscosity of feces.

[2] Five animals removed because of diarrhea.

[3] Remaining rats in poor condition.

[4] Some diarrhea.

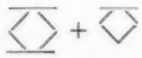
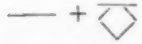
The only deaths recorded were in lots where the diets contained either non-adduct-forming monomers or dimeric or higher polymers, with the former displaying the greater toxicity. Also, in every lot receiving esters of polymeric acids there was diarrhea and the feces were varnish-like. This material was so sticky that at morning inspection the feet and tails were often found to be inseparable without washing. In some cases the animals were stuck to the wire floor of their cages. The diets, however, did not appear to be toxic in the usual sense of the term.

The ester fraction which consisted entirely of urea adduct-forming monomers had no harmful effect on survival. The one death in lot 3 may have been due to the "cyclic" monomers of linseed oil also present, since all rats died in lot 5 even where the diet contained but 10% of such material.

Gain of rats. The gain figures shown in table 4 are not directly comparable as between oils. For each ester source, however, figures are strictly comparable as between diets and between levels.

TABLE 4

Live weight gains — 28 days

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
		gm	*gm*	*gm*	*gm*	*gm*	*gm*
1	——	101	107	93	99	143	150
2		32	39	..	63	107	150
3		4	66	83	101	162	162
4	——	57	108	91	99	151	159
5		..	..	16	36	97	142
6		13	77	..	69	90	143
Least significant difference (P = 0.05)		23	23	10	10	20	20

The results leave little doubt that esters of both "cyclic" monomeric and polymeric acids from the heat polymerization of these three oils are undesirable components of rat diets. However, there would seem to be a difference in the degree of toxicity of the non-adduct-forming distillable fractions, insofar as that from sunflower seed oil was less damaging than those derived from flaxseed or soybean oil. These oils differ chiefly in their contents of the trienoic linolenic acid and there is a temptation to ascribe to this fatty acid the origin of the toxic "cyclic" monomers. Such an ascription requires an assumption that some non-adduct-forming material arises on heat polymerization from some fatty acid other than linolenic and perhaps also that this is a less toxic material than that formed from trienoic fatty acids.

Food intake. Fractions 1 and 4 were equally readily eaten, except at the 20% level of the linseed oil fractions. The polymer fraction 6 was practically equally acceptable, except at the 20% level of the linseed oil fraction; this is remarkable in view of the unattractive varnish-like nature of the materials in question. The "cyclic" monomer fraction (fraction 5) was less readily eaten than the other fractions; and here it is to be noted that these fractions were either colorless or very pale, bland oils. Fractions 2 and 3, containing some "cyclic" monomer, were as readily accepted as fractions 1 and 6, in the case of the soybean and sunflower seed oil fractions, but less readily in the case of the linseed fraction. On the whole, these results suggest that the only fraction that was definitely poorly acceptable was fraction 5.

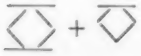
Digestibility of the oils. There was reasonably clear evidence that the non-adduct-forming polymers were poorly digestible. This was predictable from the abnormal feces. It is probable that low digestibility was a major causal factor in the slower gain of the animals fed this oil fraction. The digestibility of other fractions was above 90%.

Efficiency of utilization of dietary calories (gain per 1000 digested calories). The data suggest that, in general, the rats used that portion of the calories which they absorbed about

equally well excepting for the "cyclic polymers." In the case of the linseed and soybean oils, the digested polymeric esters were in some cases as efficient as the straight-chain materials, but with sunflower seed oil this fraction was as unsatisfactory as the cyclic monomers (table 5).

TABLE 5

Gains per 1000 digested calories

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
		gm	*gm*	*gm*	*gm*	*gm*	*gm*
1	——	61	61	99	99	82	89
2		9	50	..	88	69	101
3		43	63	92	104	95	90
4	——	54	62	99	106	92	87
5		..	..	85	85	77	93
6		61	56	..	88	65	84
Least significant difference (P = 0.05)		16	16	10	10	14	14

DISCUSSION

Certain characteristics of the non-adduct-forming distillable ester fractions from the three oils are presented in table 6. The order of decreasing injuriousness was also the order of decreasing iodine value and refractive index, while the cryoscopic mean molecular weights were, for practical purposes, the same and corresponded to a preponderance of C_{18} acids. The most marked difference between the three NAFD fractions was in respect to their behaviour on alkali isomerization (fig. 2). Linseed NAFD displayed a relatively low absorption at 233 mμ, while both soybean NAFD and sunflower seed

TABLE 6

Iodine values and refractive indices (n^{25}_d) of fractions of ethyl esters of linseed, soybean and sunflower seed oils

FRACTION		LINSEED	SOYBEAN	SUNFLOWER SEED
1. Adduct-forming fraction of total esters	Iodine no.	118.2	99	..
	n^{25}_d	1.45345	1.45302	..
2. Non-adduct-forming fraction of total esters	Iodine no.	162.7	125	..
	n^{25}_d	1.47561	1.46998	..
3. "Distillable" esters	Iodine no.	130.1	106	110
	n^{25}_d	1.45684	..	..
4. Adduct-forming fraction of "distillable" esters	Iodine no.	124.8	92	107
	n^{25}_d	1.45494	1.44935	1.45254
5. Non-adduct-forming fraction of "distillable" esters	Iodine no.	170.7	143	130
	n^{25}_d	1.46986	1.47001	1.45671
6. "Non-distillable" esters	Iodine no.	159.9	114	106
	n^{25}_d	1.48017	..	1.47655

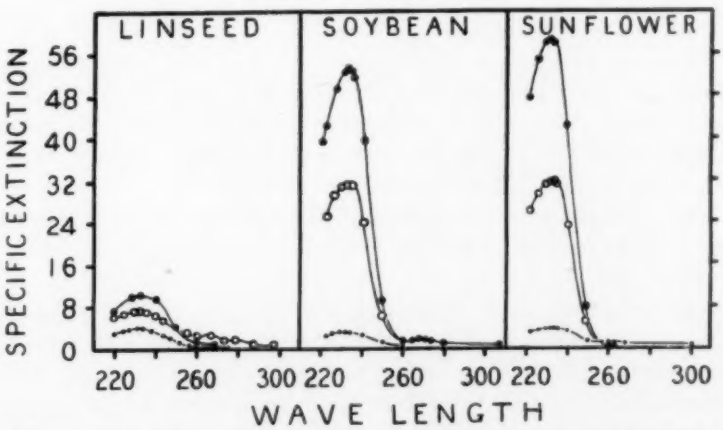


Fig. 2 Ultraviolet absorption spectra of non-adduct-forming distillable fractions of the esters of heat polymerized linseed, soybean and sunflower seed oils.

●—●—● unisomerized.
○—○—○ alkali-isomerized for 25 minutes.
●—●—● alkali-isomerized for 6 hours.

NAFD displayed a high proportion of difficultly conjugable diene unsaturation, as shown by the increase in absorption at 233 mμ on extending the isomerization time from 25 minutes to 6 hours. This increase could be explained by the presence of much *cis-trans* acid (Jackson, Paschke, Tolberg, Boyd and Wheeler, '52). The results with linseed oil suggest a much lower proportion of difficultly conjugable *cis-trans* isomer. In point of fact, oxidation with permanganate by Bertram's method showed that linseed NAFD contained less than 2% of saturated material, and the spectrometric data and iodine value suggest that linseed NAFD contained a high proportion of non-conjugable diene, and perhaps as much as 80%. But we are not yet in a position to state what feature of the chemical construction is responsible for the failure to form an urea adduct. It is, of course, possible that this feature may be related to the nutritional defectiveness of the fraction; at the same time it must be borne in mind that failure to form urea adducts may result from a variety of structural features.

SUMMARY

The non-adduct-forming fraction (NAFD) of the distillable esters from heated soybean oil was toxic, though to a lesser degree than that from the comparable fraction obtained from linseed oil. The NAFD from heated sunflower seed oil, however, was only slightly injurious to the rats.

The adduct-forming fractions from both the heated soybean oil and the heated sunflower seed oil were nutritionally harmless.

The chief chemical difference between the NAFD fractions from the three heated oils was in respect to their behaviour on alkali isomerization. The NAFD from heated linseed oil displayed relatively little increase of its absorbance at 233 mμ, whereas the results for the soybean and sunflower seed oils suggested the presence of high proportions of difficultly conjugable diene unsaturation.

These results suggest that the non-adduct-forming fraction of the distillable esters of heated linseed may contain a high

proportion of non-conjugable diene cis-isomers, possibly of cyclic structure.

ACKNOWLEDGMENTS

The authors are indebted to the Canadian Committee on Edible Fats and Oils, National Research Council of Canada, Ottawa, for financial support and for frequent helpful discussions. They wish also to thank Dr. W. D. McFarlane and Dr. W. F. Parker, Canadian Breweries Limited, Toronto, Ont., for providing the sample of soybean oil.

APPENDIX

Preparation of ethyl esters of polymerized soybean, sunflower seed and linseed oils.

Raw solvent-extracted soybean oil (Victory Mills Limited, Toronto) was alkali-refined in batches of 1.5 kg with 3.6% 20° Baumé sodium hydroxide, washed and dried with sodium sulphate. The correct amount of alkali was calculated from the acid value and the tables given by Bailey ('51). Batches of 500 gm of the oil were then bleached with 2% Super Filtrol, filtered and polymerized at 275°C. for 20 hours under a stream of CO_2. From that point the preparation was as described by Crampton et al. ('55).

Raw sunflower seed oil (Co-operative Vegetable Oils Limited, Altona, Man.) was handled in the same fashion, except that a heating time of 26 hours was selected for the polymerization. Preliminary experiments showed that there was not any appreciable formation of NAFD fraction until this oil had been heated for 20 hours. The subsequent steps in the preparation of ester fractions were performed as for the linseed and soybean oils.

The yields and mean molecular weights of the various fractions are given above in table 2. The corresponding iodine numbers and refractive indices are reported in table 6.

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DEVELOPMENT OF FATTY LIVERS DURING LACTATION OF RATS FED AMINO ACID RATIONS[1]

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(Received for publication March 22, 1956)

During earlier studies on the reproduction of rats fed protein-free amino acid rations, it was noted (Schultze, '55) that after completion of lactation the livers of rats were greatly enlarged, pale colored, mottled and friable. Since this condition was not observed in non-lactating animals of similar age fed the same rations, it appeared that fatty livers had been induced by the stress of lactation. This condition was investigated in conjunction with extended studies (Schultze, '56) of the adequacy of protein-free amino acid rations for reproduction and lactation. Moreover, since the literature does not appear to contain a record of previous systematic observations on this point, it was necessary to investigate the effect of pregnancy and lactation on the lipid content of livers of rats fed adequate rations composed largely of natural products. The results of this work are summarized in this paper.

EXPERIMENTAL

Rations. The rations contained the ingredients listed in table 1. Amino acid mixtures I and II which contained 10 and 16 amino acids respectively had the same composition as previously described (Schultze, '55) except that the isoleucine

[1] Paper no. 3513, Scientific Journal Series, Minnesota Agricultural Experiment Station.

was from a different source.[2] The rolled oats — casein ration OC_4 differed from similar rations previously described (Gander and Schultze, '55) in that unpurified commercial casein was substituted for the leached casein prepared in the laboratory. Such a ration can support very heavy lactation (Schultze, '54) and simultaneous weight gains of mothers. The rations were mixed at least once a week and stored at 4°C.

TABLE 1

Composition of rations

COMPONENT	RATION					
	AA_4	AA_{15}	AA_{16}	AA_{17}	AA_{19}	OC_4
Amino acid mixture I,[1] gm	159.4	....	....	....	....	....
Amino acid mixture II,[1] gm	....	122.3	183.4	122.3	244.5	....
Diammonium citrate, gm	51.6	....	....	....	....	....
Casein, commercial, gm	....	....	....	....	....	65.0
Rolled oats, ground, gm	....	....	....	....	....	840.0
Sucrose + B vitamin mixture I,[2] gm	20.0	20.0	20.0	20.0	20.0	20.0
Salt mixture IV,[3] gm	40.0	40.0	40.0	40.0	40.0	30.0
Sucrose, gm	699.0	787.7	726.6	687.7	665.5	11.7
Hydrogenated vegetable oil,[4] gm	....	....	....	100.0	....	....
Wheat germ oil, gm	20.0	20.0	20.0	20.0	20.0	20.0
Corn oil + vitamins A and D_2,[2] gm	10.0	10.0	10.0	10.0	10.0	10.0
DL-Methionine, gm	....	....	....	....	....	3.3
Pyridoxine hydrochloride, mg	5	....	....	....	....	....
Total grams	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[1] Schultze, '55.
[2] Gander and Schultze, '55.
[3] Schultze, '50.
[4] Crisco.

Animals. All rats were from our black strain of line 3 which had been used in earlier studies (Schultze, '55). They were housed in groups of 5 in cages provided with raised screens except shortly before parturition and during lactation, when a cage, bedded with clean wood shavings, was provided for each animal.

Rats fed the rolled oats — casein ration. Littermate female rats, three weeks old, whose mothers had consumed the same

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

ration were alloted at random to 7 groups and fed ration OC_4 throughout the experiment. The rats in groups 1a, 1b, and 1c, table 2, were not bred; all others were bred at 10 weeks of age. The rats in group 1a were killed when their littermates were bred; those in group 1b at parturition of their littermates, and those of group 1c at the end of a three weeks period of lactation of their littermates. The rats in group 2 were killed within 7 hours after birth of their young. Within 12 hours after parturition, the number of young nursed by rats in group 3a was reduced to two; in group 3b to 6, and in group 3c it was increased to 12, where required, by the addition of young from groups 3a or 3b. When any nurslings died, they were replaced by rats of about the same age obtained from litters which were raised for this purpose. Thus it was possible to compare the lipid content of the livers of rats at the end of pregnancy or at the end of a lactation period of varying degree of intensity with that of non-lactating littermates of the same age. The rats in groups 3a, 3b, and 3c were killed after a period of lactation of 21 days.

Rats fed protein-free amino acid rations. These animals were offspring from mothers fed the same or similar protein-free rations. With the exception of group 8, table 3, fed ration AA_{17}, each group was fed the same ration from weaning until killed. Group 8 was transferred from ration AA_{13} to AA_{17} after the second litters had been weaned. All animals in groups 2, 5, 7, and 9, table 3, were killed on the 28th day of their second lactation; those in group 8 on the 28th day of their third lactation; those in groups 3 and 6 were killed 14 days after completion of their second lactation period of 28 days. The size of the litters was not changed by addition or removal of young after parturition and the mean number of young nursed for 28 days ranged from 6.0 to 7.3 per mother in all groups except 1, 4 and 10. The latter three included a few animals which lost their litters through abortion or shortly after birth, i.e. all rats in these groups were subjected to no stress

of lactation or to a very light one. These rats were killed when their litters died or after lactating for 28 days.

Determination of liver lipids. The livers were removed from the decapitated rats, weighed and partially dried at 60° C. The whole liver was then macerated and representative samples were used for analysis of residual moisture and of lipids by a procedure previously described (Hedin and Schultze, '55).

TABLE 2

Effect of pregnancy and lactation on liver lipids[1]

GROUP	REPRODUCTIVE STATUS[2]	BODY WT.	LIVER		
			Per cent of body weight	Dry weight	Lipids
		gm		*% of fresh*	*% of dry weight*
1a	N.P.	171 ± 4.7[3]	3.88 ± 0.09	29.6 ± 0.37	21.6 ± 0.60
1b	N.P.	205 ± 6.2	3.62 ± 0.26	29.0 ± 0.78	23.0 ± 0.85
1c	N.P.	207 ± 3.8	3.42 ± 0.07	28.9 ± 0.33	21.5 ± 0.68
2	P	221 ± 5.5	3.89 ± 0.05	27.4 ± 0.27	24.7 ± 0.58
3a	N_2	247 ± 6.5	3.85 ± 0.10	28.2 ± 0.59	20.8 ± 1.05
3b	N_6	235 ± 3.4	4.92 ± 0.19	28.9 ± 0.30	20.3 ± 0.33
3c	N_{12}	236 ± 3.6	4.88 ± 0.10	28.0 ± 0.25	21.4 ± 0.92

[1] Means of 10 animals except group 3c which comprised 14 animals; all rats fed ration OC_4.

[2] N.P. = not pregnant; P = killed after parturition; N_2, N_6, N_{12} = killed after nursing 2, 6, 12 young respectively for 21 days.

[3] Standard error of the mean.

RESULTS AND DISCUSSION

Lipid content of livers of rats fed a natural ration. The results obtained with the rolled oats — casein ration are summarized in table 2. Comparison of the data from groups 1a, 1b and 2 indicate that during pregnancy there was only a slight, statistically non-significant ($P = > 0.10$) increase in the lipid content of the livers. During lactation, however, the lipid content of the livers remained in the range of values found in non-lactating littermates of the same age (compare groups 3a, 3b, and 3c with group 1c). If it is assumed that the lactating rats at parturition had the same content of liver lipids as their littermates (group 2) which were killed shortly

after parturition, there was in all three lactating groups a statistically significant decrease in concentration of liver lipids; (comparing groups 3a and 3b with group 2, $P = < 0.01$; comparing group 3c with group 2, $P = < 0.02$). Evidently even the heaviest stress of lactation, when supported by an adequate diet did not induce fatty livers. In the mothers which nursed 6 or 12 young, there was, however, a significant ($P = < 0.01$) increase in the mass of the liver, not accounted for by increased proportions of water and lipid. The livers of all groups had essentially the same concentration of solids. Even during the heaviest lactation the mothers gained weight. Poo et al. ('39) observed that the weight of the livers of rats increased during pregnancy but not during lactation whereas Field et al. ('42) found an increase both in pregnancy and lactation. Beauvallet ('53) observed fatty livers in a small proportion of rats killed at the end of lactation. Beaton et al. ('54) found that the absolute size of the liver increased during pregnancy of rats.

The amount and composition of the diet consumed can have a profound effect on the liver fat in pregnancy. Thus, Ferguson ('54) observed that pregnant sheep fed on a high plane of nutrition had normal livers whereas those on a low plane of nutrition developed fatty livers, associated sometimes with toxemia of pregnancy (Parry, '54). Himsworth ('47) found that the stress of pregnancy can induce gross fatty infiltration of livers of rats fed diets which contain just sufficient choline and casein to prevent fat accumulation in non-pregnant animals. Instances of fatty and enlarged livers associated with pregnancy have also been observed among Africans subsisting on diets deficient in protein (Woodruff, '51).

Lipid content of livers of rats fed protein-free amino acid rations. Table 3 presents a summary of the results obtained with 5 different protein-free rations. In young growing rats, rations AA_4 and AA_{15} produced a temporary accumulation of liver fat [3] but as the animals became older the lipid con-

[3] Hallanger and Schultze, unpublished data.

TABLE 3

Effect of lactation on lipid content of livers of rats fed amino acid rations

GROUP	RATION	NUMBER OF RATS	YOUNG NURSED PER LITTER	LIVER REMOVED ON DAY	BODY WEIGHT	LIVER		
						Per cent of body weight	Dry weight	Lipids
					gm		% of fresh	% of dry weight
1	AA_4	7	0–2	[1]	177 ± 9.5	4.55 ± 0.22	27.6 ± 1.43	25.5 ± 1.09
2	AA_4	19	> 3	P + 28 [2]	164 ± 4.1	11.27 ± 0.61	44.6 ± 2.2	66.1 ± 1.67
3	AA_4	8	> 3	P + 42 [3]	193 ± 5.1	5.86 ± 0.40	37.6 ± 1.25	46.3 ± 3.71
4	AA_{25}	5	0–2	[1]	223 + 1.8	4.23 ± 0.06	26.9 ± 1.46	28.3 ± 2.41
5	AA_{25}	16	> 3	P + 28	177 ± 4.9	5.89 ± 0.17	34.0 ± 0.69	41.9 ± 2.23
6	AA_{25}	10	> 3	P + 42	198 ± 6.8	4.06 ± 0.09	30.5 ± 0.43	29.4 ± 1.18
7	AA_{38}	9	> 3	P + 28	212 ± 4.3	7.08 ± 0.27	39.5 ± 1.71	55.8 ± 2.94
8	AA_{17}	6	> 3	P + 28	228 ± 12.0	6.37 ± 0.46	36.9 ± 1.5	45.8 ± 3.5
9	AA_{20}	8	> 3	P + 28	220 ± 14.5	6.39 ± 0.37	36.2 ± 2.2	49.9 ± 3.7
10	AA_{20}	3	0–2	[1]	226	3.94	27.7	25.3

[1] After abortion, death of litter or 28 days after birth of 1 to 2 young.
[2] P + 28 = 28th day after parturition, the day when young were weaned.
[3] P + 42 = 42nd day after parturition, 14 days after young were weaned.

centration was only slightly higher than that found in rats fed an adequate ration. The results obtained with groups 1, 4 and 10, table 3, which included rats that aborted, lost their young shortly after birth, or nursed only one or two young indicate that during pregnancy a marked increase of the lipid content of the liver had not occurred. In contrast to this, however, all groups which were subjected to a strong stress of lactation and then killed, showed an increase in the size and particularly in the lipid content of the livers. When the diet contained only 10 amino acids (group 2), this increase was particularly striking even though the amino acids were furnished at a higher level than in ration AA_{15} containing 16 amino acids (group 5). An increase in the fat and energy content of the ration did not prevent fatty infiltration of the livers (compare groups 5 and 8) while an increase in the amino acid content from 12.2 (group 5) to 18.3% (group 7) increased the relative size of the livers and their lipid content. A further increase of the amino acid content of the ration to 24.4% failed to prevent enlargement or fatty infiltration of the livers (group 9). With these diets the hypertrophy and fatty infiltration of the liver is clearly the result of the stress of lactation because within two weeks after weaning, the size and fat content of the liver decreased towards normal levels as indicated by comparing groups 2 and 5 with groups 3 and 6 respectively. Coincident with the increase in fat content of the livers there was also an increase in the absolute and relative amounts of non-fat solids, presumably protein. The moisture content of the livers decreased, however, with increasing liver fat. The temporary hypertrophy and fatty infiltration of the livers apparently did not inflict permanent damage because many rats fed the same rations had several successive pregnancies and lactations and their young had a very low rate of mortality (Schultze, '56).

The reason for the development of fatty livers during lactation is not clear at present. Doubling of the methionine content of the ration did not prevent the condition, which may

be related to a physiological imbalance of the ratios of the amino acids (Harper et al., '54) although the "essential amino acids" in rations AA_{15}, AA_{16}, AA_{17} and AA_{19} were present in relative proportions similar to those found in casein. The concentration of the lipotropic factors, methionine and choline, in these diets was sufficient to prevent fatty livers in mature rats which were not lactating. The development of severely fatty livers during lactation illustrates strikingly that a ration which is satisfactory for the growing or mature animal may be quite inadequate to prevent pathologic changes in tissues when the metabolic economy of the organism is subjected to temporary severe stress.

SUMMARY

1. Rats fed protein-free amino acid rations developed hypertrophied and severely fatty livers under the stress of lactation. After lactation the concentration of liver lipids decreased.

2. When lactation of rats was supported by an adequate diet some hypertrophy of the liver was observed but the lipid content of the liver decreased.

ACKNOWLEDGMENTS

Financial support for part of this work from the General Research Fund of the Graduate School, University of Minnesota, and from the Williams-Waterman Fund of the Research Corporation, New York, is gratefully acknowledged. The L-lysine hydrochloride used was a gift from E. I. DuPont de Nemours and Co. The B vitamins were gifts generously furnished by Merck and Co. and the Lederle Laboratories.

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REPRODUCTION OF RATS FED PROTEIN-FREE AMINO ACID RATIONS [1]

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(Received for publication March 22, 1956)

It was previously reported (Schultze, '55) that rats fed protein-free rations containing 16% of a mixture of the 10 "essential amino acids" or 12.2% of a mixture of 16 amino acids could rear their young with a relatively low mortality. The young, however, had subnormal weaning weights and the mothers lost much weight during lactation. In addition, the young born to mothers fed the amino acid rations failed to grow at a normal rate even though suckled by foster mothers fed a natural ration. It appeared that the rations then used were qualitatively or quantitatively inadequate to support satisfactory lactation. Although a few animals of the F_2 generation of rats fed protein-free rations reared their young, there was no assurance that successful reproduction and lactation could be supported by such rations for further generations.

This work was, therefore, extended through the F_4 generation. Moreover, the adequacy for reproduction and lactation of rations containing different levels of 16 amino acids was studied through three generations and comparisons were made with a ration containing 11 amino acids.

EXPERIMENTAL

Animals. The strain of rats and their management were the same as previously reported (Schultze, '55). Some of

[1] Paper no. 3512 Scientific Journal Series, Minnesota Agricultural Experiment Station.

the rats were offspring from mothers fed protein-free amino acid rations, as indicated in the tables, while those identified as P generation were born to mothers fed a ration containing 84% of rolled oats and 6.5% of crude casein and the mixture of lipids, salts, and vitamins described elsewhere (Hallanger and Schultze, '56). These rats were weaned when 21 days old and then fed the amino acid rations while those born to mothers fed the amino acid rations were weaned at 28 days of age. The size of the litters was never reduced. The mothers were housed with males immediately after weaning the first litter.

Rations. The composition of the amino acid mixtures I and II,[2] containing 10 and 16 amino acids respectively, has been previously reported (Schultze, '55). Mixture III, containing 11 amino acids, was patterned after mixture II with the following modifications: (1) glycine, DL-alanine and DL-aspartic acid were replaced by equimolar quantities of L-glutamic acid; (2) L-tyrosine was replaced by an equimolar quantity of DL-phenylalanine; (3) L-cystine was replaced by two times its molar quantity of DL-methionine. With these mixtures, the rations were compounded as shown in table 1 of the preceding paper (Hallanger and Schultze, '56). Ration AA_{18} contained, per kilogram, 191.2 gm of amino acid mixture III, 718.8 gm of sucrose and the salt, vitamin and lipid components of ration AA_{16}.

RESULTS AND DISCUSSION

Reproduction and lactation of rats fed 10 amino acids. Table 1 includes a summary of the observations made with the F_3 (group 1) and the F_4 (group 2) generations of rats fed ration AA_4 containing 15.9% of a mixture of the "essential amino acids" plus 5.2% of diammonium citrate. Of 25 rats, only one failed to conceive; the others delivered 42 litters with a mean of 6.1 young. There was one abortion and two rats died shortly before parturition. The weight gains during

[2] The isoleucine was stated by the manufacturer to contain L-isoleucine, 50%, with D-alloisoleucine, 50%.

TABLE 1

Reproductive performance of rats fed rations with 10 or 11 amino acids

CATEGORY OF INTEREST	GROUP 1		GROUP 2		GROUP 3	
Ration; % "essential" amino acids in ration	AA_4; 15.94		AA_4; 15.94		AA_{10}; 12.80	
Generation fed amino acid rations	F_2 [1]		F_4 [2]		F_2 [3]	
Pregnancy	1st	2nd	1st	2nd	1st	2nd
Number of mothers; number of litters born alive	12;12	12;11	13;10	10;9	11;10	8;8
Mean weight gain during pregnancy, gm	38.4	41.5	54.7	63.2	79.4	71.0
Total number of young born at term	65	59	79	65	86	63
Mean number of young per litter weaned	5.3	5.4	7.9	7.1	8.5	7.5
Mortality of young in 28 days, %	35.4	8.5	0	1.5	10.4	4.8
Mean weight of young, 28 days old, gm	30.8	33.0	20.5	28.1	34.7	37.1
Mean weight loss of mothers, first 7 days, gm	8.1	10.0	10.4	13.6	10.0	7.5
Mean weight loss of mothers, 28 days, gm	20.6	20.9	13.8	21.5	19.4	16.6

[1] Offspring of F_2 generation rats previously described (Schultze, '55, p. 571).
[2] Offspring of group 1.
[3] Offspring of group 8 and littermates of groups 9 and 10, table 3.

TABLE 2

Reproductive performance of rats fed ration AA_{15} containing 12.2% of 16 amino acids

CATEGORY OF INTEREST	GROUP 4		GROUP 5		GROUP 6	
Generation fed amino acid ration	P [1]		F_1 [2]		F_2 [3]	
Pregnancy	1st	2nd	1st	2nd	1st	2nd
Number of mothers; number of litters born alive	12;12	10;9	24;23	19;17	12;12	12;11
Mean weight gain during pregnancy, gm	64.7	62.1	59.1	71.8	67.5	63.4
Total number of young born at term	80	64	141	112	84	63
Mean number of young per litter weaned	6.5	6.9	6.3	7.0	6.6	5.1
Mortality of young in 28 days, %	2.5	25.0	13.1	8.9	3.6	11.1
Mean weight of young, 28 days old, gm	28.2	32.5	30.2	31.5	25.1	33.4
Mean weight loss of mothers, first 7 days, gm	20.5	18.1	19.0	18.6	21.8	17.8
Mean weight loss of mothers, 28 days, gm	29.7	35.8	26.1	30.3	25.3	20.5

[1] The rats identified as P generation were born to mothers fed a ration of 84% of rolled oats, and 6.5% of crude casein and the mixture of lipids, salts and vitamins described by Hallanger and Schultze ('56).
[2] Offspring of group 4.
[3] Offspring of group 5.

the pregnancy were below normal particularly in the F_3 generation in which only 5.4 young were born per litter. Except in the first litters of the F_3 generation the mortality of the young was low. Their mean 28-day weight gains were small, particularly among the larger litters (group 2) and the mean total weight of the litters at that time ranged from 162 to 198 gm. Since some of the young, in small litters, reached a 28-day weight of 51 gm, the limited weight gains of most of the suckling rats appear to be due to insufficient amounts of milk secreted by the mother. There was no evidence of gradual deterioration of reproduction and lactation with successive litters or generations.

Reproduction and lactation of rats fed 11 amino acids. The total amount of the "essential amino acids" and other sources of nitrogen in the ration are not necessarily the main determinant of the nutritive value of protein-free rations. This is demonstrated by the results obtained with group 3, table 1, which was fed, after weaning, a ration containing 12.8% of a mixture of the "10 essential amino acids" plus 6.3% of L-glutamic acid as a source of additional nitrogen. These animals made greater weight gains during pregnancy, they bore and raised larger litters and the mean total weight (287 gm) of the litters at 28 days of age was 58% greater than that (176 gm) of the litters of mothers in groups 1 and 2 whose ration contained 15.9% of the "essential amino acids." This may be due to more effective utilization of the nitrogen from L-glutamic acid than from diammonium citrate although the total amount of nitrogen contributed by amino acids was similar in both cases, 2.02% in ration AA_4 and 2.19% in ration AA_{18}. It is more likely that the relative proportions of the various amino acids in ration AA_{18} were more suitable. The molar ratios of the various amino acids to tryptophan (see Flodin, '53) in rations AA_4 and AA_{18} are respectively: arginine 1.17 and 2.67; histidine 2.65 and 1.99; isoleucine 7.79 and 11.2; leucine 6.23 and 10.64; methionine 4.11 and 5.79; phenylalanine 5.57 and 7.58; threonine 8.58 and 7.42; valine 12.2 and 13.6; lysine 7.23 and 4.6. Of these, the relative

amounts of methionine and phenylalanine appear to be of minor importance because in ration AA_{16} which supported a performance similar to ration AA_{18}, the corresponding molar ratios were 3.62 and 4.84 respectively. Since the absolute amounts of histidine, isoleucine, methionine, threonine, phenylalanine, tryptophan, valine and lysine are greater in ration AA_4 than in ration AA_{18}, while those of arginine and leucine are 35 and 16% smaller respectively, it appears that a qualitatively "better balance" among the various amino acids is primarily responsible for the better reproduction and lactation supported by the amino acid mixture III.

Reproduction and lactation of rats fed 12.2% of a mixture of 16 amino acids. Previous observations with a few animals (Schultze, '55, table 4, group 8) had suggested that rats born to mothers fed 12.2% of a mixture of 16 amino acids incurred a very low mortality. Repetition and extension of this work with larger numbers of animals, covering two litters each from a parental and two successive filial generations confirm the preliminary observations as summarized in table 2. In comparison with groups 1 and 2 (table 1) the weight gain of the mothers during pregnancy was in most instances higher; the weaning weights of the young were essentially the same but the loss of weight of the mothers during lactation was much greater. Most of the weight loss occurred during the first 7 days after parturition at a time when milk production is not at a maximum. The mean weight gains of the second litters were always greater than those of the first litters. There was no evidence in successive pregnancies or generations of a progressive deterioration of the ability to reproduce or lactate.

Although ration AA_{15} contained only 7.47% of the "essential amino acids," the reproductive performance and lactation were not inferior to those observed with ration AA_4 which contained 15.9% of the same amino acids. The mean total 28-day weight of the litters from all groups fed ration AA_{15} was 194 gm compared to 176 gm observed with ration AA_4.

This again suggests that the "balance" of the amino acids was better in the mixture II.

The effect of increasing the amino acid content of the ration. Since the small weight gains of the young and the large weight losses of the mothers during lactation may have been the result of a suboptimal intake of amino acids, the effect of increasing the amino acid content was investigated with 4 groups of rats. During growth, the first pregnancy and lactation group 7 (table 3) was fed ration AA_{15}; during this time the animals represented a replication of the experiments with group 4 (table 2) and the results obtained were very similar. Immediately after weaning of the first litter and throughout the second pregnancy and lactation, the rats of group 7 were fed ration AA_{16} which contained 50% more of the amino acid mixture II. During the second pregnancy, these rats made greater weight gains, their young attained about 50% greater weaning weights and the weight losses of the mothers were smaller. This superior reproductive and lactation performance was maintained through two filial generations, namely in groups 8 and 9 (table 3). Although the mean 28-day weight of the young of the F_2 generation was smaller than observed in the F_1 generation (group 8), this cannot be interpreted as a progressive deterioration of the lactation with successive generations because the mean total weight of the litters in both pregnancies of each group was essentially the same, 300 to 329 gm. When the amino acid content of the ration was increased to 24.4% (group 10, table 3) the nutritive value of the ration was not further increased as measured by the weight gains during pregnancy, survival and total mean total weight of the litters at weaning (290 to 299 gm) or the weight change of the mothers during lactation. The number of litters available in these experiments was too small for valid application of more critical methods for evaluation of lactation (Schultze, '54).

The effect of five non-essential amino acids on reproduction and lactation. A comparison of the results obtained with group 3 (table 1) and group 9 (table 3) which were comprised

TABLE 3

Reproductive performance of rats fed rations containing 12.2 to 24.4% of 16 amino acids

CATEGORY OF INTEREST	GROUP 7		GROUP 8		GROUP 9 [1]		GROUP 10 [1]	
Ration; % amino acids	AA_{12}; 12.2	AA_{16}; 18.3	AA_{16}; 18.3		AA_{16}; 18.3		AA_{16}; 24.4	
Generation fed amino acid ration	P [2]		F_1 [3]		F_2 [4]		F_2 [4]	
Pregnancy	1st	2nd	1st	2nd	1st	2nd	1st	2nd [5]
Number of mothers; number of litters born alive	11;11	11;11	10;10	10;10	9;9	9;9	10;10	10;9
Mean weight gain during pregnancy, gm	59.5	75.9	74.8	79.4	76.8	73.1	70.8	65.4
Total number of young born at term	78	80	83	81	80	68	78	66
Mean number of young per litter weaned	7.0	7.3	7.9	6.9	8.7	7.4	7.3	6.4
Mortality of young in 28 days, %	1.3	7.6	4.8	13.4	2.5	1.5	4.3	1.7
Mean weight of young, 28 days old, gm	28.7	43.4	41.7	46.1	36.2	40.6	39.7	46.7
Mean weight change of mothers, 7 days, gm	−20.7	−12.4	−2.2	−6.0	−2.1	−4.3	−3.6	+1.2
Mean weight change of mothers, 28 days, gm	−28.4	−15.0	+4.9	−3.2	+3.1	−5.3	−0.9	+1.4

[1] Animals in groups 9 and 10 were littermates of those of group 3, table 1.

[2] The rats identified as P generation were born to mothers fed a ration of 84% of rolled oats and 6.5% of crude casein and the mixture of lipids, salts and vitamin described by Hallanger and Schultze ('56).

[3] Offspring of group 7, second litters.

[4] Offspring of group 8.

[5] Includes third litter of one rat which aborted the second litter.

of littermates permits evaluation of the combined effects of alanine, aspartic acid, tyrosine, glycine and cystine on the nutritive properties of the ration. The rations of both groups were identical with respect to total nitrogen and the amounts and proportions of 8 of the "essential amino acids"; in the ration of group 3, L-glutamic acid was substituted for equimolar quantities of alanine, glycine, and aspartic acid; phenylalanine was similarly substituted for tyrosine and two moles of methionine replaced one mole of cystine in the ration of group 9. In both groups, the mortality of the young was low, there was no significant difference in weight gains during pregnancy or of the suckling young of the first litters which contained about the same mean number of young weaned in both groups. The second litters in group 9, however, reached significantly higher ($P < 0.01$) weights in 28 days. In addition, the mothers in group 9 lost much less weight than those in group 3. The presence of glycine, alanine, aspartic acid, cystine, and tyrosine in the diet appeared to support somewhat better lactation and eased its stress on the mother.

The high percentage of young weaned during several generations fails to support the view (Moruzzi et al., '54; Piccioni et al., '51) that unknown compounds associated with proteins are essential for survival of the young rat.

The effect of increased lipid content on the ration. Rose et al. ('54) have shown that young men fed amino acid diets require a high caloric intake for the maintenance of nitrogen equilibrium. Since the rations used in the present and preceding (Schultze, '55) studies contained only 3% of a lipid component, 6 rats from group 5 were fed, immediately after birth of the third litters, ration AA_{17} in which 100 gm of hydrogenated vegetable fat [3] replaced 100 gm of sucrose per kilogram of ration AA_{15}. All of the 40 young born survived and reached a mean 28-day weight of 31.0 gm compared to 30.2 and 31.5 gm attained by the young of the first two litters. During lactation the mothers lost a mean of 25.8 and 39.3 gm in 7 and 28 days respectively. The addition of fat and the

[3] Crisco.

increased caloric density failed, therefore, to support better lactation or to ease its stress.

Post-weaning weight gains. None of the rations supported maximum post-weaning weight gains. When an adequate natural ration is fed to this strain of rats, the females gain about 120 gm, the males about 180 gm in 6 weeks after weaning. The mean weight increments in grams in 6 weeks of the various groups fed amino acid rations were: group 1, 65.0; group 2, 82.6; group 3, 93.4; group 4, 109; group 5, 95.2; group 6, 88.6; group 7, 105; group 8, 95.1; group 9, 104; group 10, 98.2.

Among the rats fed the 12.2% amino acid rations, those of groups 4 and 7 whose mothers received the natural ration made significantly greater weight gains ($P < 0.02$) than those of groups 5 and 6 whose mothers consumed amino acid rations. The best comparison of the growth response can be made with groups 3, 9, and 10 which were comprised of littermates. The rats fed a ration containing 18.3% of a mixture of 16 amino acids (group 9), made 6-week post-weaning weight gains that were significantly greater ($P < 0.05$) than those receiving only 11 amino acids (group 3). The weight gains of group 10 fed 24.4% of a mixture of 16 amino acids were intermediate between those of the other two groups, but not significantly different.

Similar comparisons were also made with groups of 9 to 10 males that were littermates of the females of groups 3, 9, and 10. Those fed ration AA_{16} and AA_{19} made the same mean 6-week gains, 147 and 146 gm, while those fed ration AA_{18} gained only 129 gm, a significantly smaller amount ($P < 0.01$). Although young rats consuming the amino acid rations directly did not consistently increase their daily weight by more than 2.5 gm (females) to 3.5 gm (males), many litters had total daily weight increaments of more than 10 gm throughout the period when they subsisted solely on the milk of mothers fed amino acid rations. Under the proper stimulus for food consumption, such as induced by lactation, these rations can, therefore, support far greater synthetic activity in the organism that is apparent from the weight gains of the individual.

Other observations. The difference in tables 1 to 3 between the number of mothers and the number of litters born alive in each pregnancy is due to the occurrence of a few resorptions, abortions or maternal deaths shortly before or after parturition or to failures of the animals to conceive. The incidence of such occurrences was highest, 5 out of 47 cases, in groups 1 and 2; in groups 4 to 6, it was reduced to 5/89; in groups 7 to 9, it was 0/60; and in group 10 there was one abortion. Excluding abortions, the number of young born dead was very small in all groups. The mean weights of the young at birth in groups 1 and 2 were 4.8 gm, in the other groups 5.0 gm.

With all of the amino acid rations, the livers of the mothers became greatly enlarged and very fatty during lactation. The quantitative aspects of this observation will be reported separately (Hallanger and Schultze, '56).

SUMMARY

1. Protein-free rations containing mixtures of 10, 11 or 16 amino acids were fed to rats during growth, two pregnancies, and lactation, in one instance for as long as 4 successive filial generations without evidence of gradual deterioration of the reproductive or lactation performance.

2. A ration containing 12.2% of mixture of 16 amino acids supported better lactation than a ration containing 15.9% of a mixture of the 10 "essential amino acids."

3. Lactation was better when the level of the mixture of 16 amino acids was increased from 12.2 to 18.3% but a further increase to 24.4% failed to improve reproduction or lactation.

4. A dietary supply of glycine, alanine, aspartic acid, tyrosine and cystine was not essential for reproduction or lactation but when they were fed the loss of weight of lactating mothers was greatly decreased.

5. The nutritive value of a protein-free ration is affected by the relative proportions in which the individual amino acids are present as well as by the absolute amounts.

6. None of the rations used was completely adequate for optimum preweaning or postweaning weight gains of the young or for the prevention of fatty livers during lactation.

ACKNOWLEDGMENTS

Financial support for part of this work from the General Research Fund of the Graduate School, University of Minnesota, and from the Williams-Waterman Fund of the Research Corporation, New York, is gratefuly acknowledged.

The L-lysine hydrochloride used was a gift from E. I. DuPont De Nemours and Co., through the courtesy of Dr. N. W. Flodin. The B vitamins were gifts generously furnished by Merck and Co. and the Lederle Laboratories.

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UTILIZATION OF D-TRYPTOPHAN BY THE CHICK[1]

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(Received for publication March 31, 1956)

Little agreement exists in the literature regarding D-tryptophan utilization by the chick. In 1944 Grau and Almquist reported that chicks could not utilize D-tryptophan. Wilkening et al. ('47) suggested that some of the D isomer was available to the chick and in 1947 Wilkening and Schweigert reported the utilization of D-tryptophan to be from 17 to 40%. Anderson et al. ('50) reported that, when glucose was the carbohydrate in a niacin-deficient diet, D-tryptophan was not utilized. When starch served as the carbohydrate source, however, utilization of the D isomer occurred. It was postulated by these workers that the use of starch permitted a type of intestinal microflora which could invert the D form. Wilkening et al. ('47) and Wilkening and Schweigert ('47) used starch as the source of carbohydrate. Almquist ('52) has suggested, on the basis of the work of Anderson et al. ('50) that the original report (Grau and Almquist, '44) was correct in indicating no utilization of D-tryptophan by the chick inasmuch as conversion by the microflora can scarcely be considered utilization of the isomer by the animal body. West et al. ('52) reported that both isomers of tryptophan were equally well utilized by the chick. However, these investigators supplied such a very small amount of the total tryptophan as the D isomer that much doubt is cast upon the validity of their conclusions.

[1] The data reported in this paper are taken from a thesis submitted to the Graduate College of the University of Illinois by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition.

In every instance in which D-tryptophan has been studied in the chick, the DL mixture has been used as the source of the D isomer. In the case of an amino acid whose D isomer is poorly utilized, growth experiments are not sufficiently sensitive to demonstrate differences when the DL mixture is used. This may explain much of the discrepancy which is apparent in the literature.

TABLE 1

Composition of basal diet

CONSTITUENT	AMOUNT
	%
Corn, ground yellow	60.92
Casein, hydrolyzed [1]	18.00
Gelatin (technical grade)	12.24
Corn oil, refined	3.00
Salts [2]	5.34
Choline Cl	.20
DL-Methionine	.30
Vitamins [3]	+
Total	100.00

[1] Hy-case, a salt-free product of Sheffield Chemical Company, Inc., Norwich, New York, devoid of tryptophan.

[2] Glista ('51).

[3] Vitamins (mg/kg diet): Thiamine-HCl 100; nicotinic acid 200; riboflavin 16.0; Ca-pantothenate 20.0; vitamin B_{12} 0.02; pyridoxine-HCl 6.0; biotin 0.60; folic acid 4.0; inositol 100; para-aminobenzoic acid 2.0; menadione 5.0; ascorbic acid 250; alpha tocopherol acetate 20.0; vitamin A acetate 10,000 I.U.; vitamin D_3 600 I.C.U.

The study reported herein was undertaken in an attempt to clarify further the extent to which D-tryptophan is utilized by the chick.

EXPERIMENTAL

The diet used (table 1) contained hydrolyzed casein [2] and gelatin, as the major protein sources, at the same levels as employed by Snyder ('54). The use of ground yellow corn as the carbohydrate source stemmed from the results of repeated trials in which dextrin, cerelose and ground yellow corn

[2] See footnote 1, table 1, for a description of this product.

were used singly and in combination. In every instance the use of corn as the carbohydrate source promoted superior growth. The reason for the superior performance of the corn diet is not evident. Apparently corn adds something to this diet which is otherwise lacking but whether this is an unidentified factor, an improvement in palatability, an improved amino acid balance or some other factor is not clear. This diet contains approximately 0.036% of tryptophan.

Male chicks originating from a mating of New Hampshire males to Columbian females were used in all trials. They were housed in battery brooders equipped with raised wire floors. Feed and water were supplied ad libitum. In experiments 1 to 4 inclusive, tryptophan was added to the basal diet at the expense of corn. These chicks were placed on the experimental diets immediately after hatching. The chicks injected with tryptophan (experiments 5 to 8) were reared on a complete natural-type diet for 14 days, then weighed and randomly allotted to the various treatments by weight. They were then fasted for 12 hours, fed the tryptophan-deficient diet for 72 hours and then subjected to the various treatments to which they had been allotted earlier. The injections were given subcutaneously in the neck region every three hours, with the exception of the 3:00 A.M. injection. Thus the daily allotment of tryptophan was provided by 7 injections. Physiological saline was used as the carrier.

In order to establish that L-tryptophan was not a contaminant of the samples of D-tryptophan [3] used, two different analyses were performed. These were rotation of plane-polarized light and a microbiological assay using *S. faecalis*.

Oral administration of tryptophan. The initial experiment was designed to provide information which would serve as a guide for later experiments. Accordingly the basal diet was supplemented with 0.2, 0.4 and 0.6% of D-tryptophan. If D-tryptophan were not used, no growth would be expected; if

[3] The authors are grateful to Monsanto Chemical Company, who, through the courtesey of Dr. Kenneth Maddy, provided most of the tryptophan used in this study.

utilized at approximately 40% of the L isomer, optimum growth would be expected at between 0.4 and 0.6%; if utilized as efficiently as the L isomer the 0.2 level would be expected to give optimum growth. None of these possible results materialized (table 2). Instead, only slight growth resulted at

TABLE 2

Growth of chicks fed D- *or* DL-*tryptophan*

EXPERIMENT NO.	GROUP	SUPPLEMENT TO BASAL	AVERAGE WT.	
			7 days	14 days
			gm	*gm*
	1	0.2% D-tryptophan	48	50
1 (5)[1]	2	0.4% D-tryptophan	54	63
	3	0.6% D-tryptophan	58	73
				16 days
	1	None	47	51
	2	0.2% D-tryptophan	51	58
	3	0.4% D-tryptophan	50	62
	4	0.6% D-tryptophan	57	75
2 (5)	5	0.8% D-tryptophan	64	97
	6	1.2% D-tryptophan	67	105
	7	1.6% D-tryptophan	61	116
	8	0.4% DL-tryptophan	72	166
				14 days
	1	None	45	47
	2	1.8% D-tryptophan	71	124
	3	2.1% D-tryptophan	68	131
3 (5)	4	2.4% D-tryptophan	60	124
	5	2.7% D-tryptophan	65	126
	6	0.4% DL-tryptophan	65	144
	1	None	..	48
4 (15)	2	2.1% D-tryptophan	..	134
	3	0.4% DL-tryptophan	..	133

[1] Figures within parentheses indicate the number of chicks per experimental group.

the 0.2% level and while the 0.4 and 0.6% levels caused stepwise increases, growth on the 0.6% level was still far below a normal value.

When these points were plotted they fell on a straight line and analysis of variance revealed the regression sum squares

to be highly significant. Least squares calculations and extrapolation revealed that a chick weighing 140 gm at two weeks would require 1.8% of D-tryptophan. Since only three points were available, and since the line was extended beyond the data, the 1.8% value can be regarded with some skepticism.

In the second experiment D-tryptophan was supplied at 0.0, 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6% and a positive control receiving 0.4% DL-tryptophan was included. Once again the graded response noted in the first experiment was evident. The fitted line had a highly significant regression sum squares and extending the line indicated that 2.6% of D-tryptophan would be necessary in order to demonstrate growth equal to that obtained with DL-tryptophan.

In the third experiment D-tryptophan was present at 0.0, 1.8, 2.1, 2.4 and 2.7% of the diet. The results show that maximum growth was obtained when D-tryptophan was provided at 2.1% of the diet. It is evident, however, that the differences between the 4 lots supplemented with D-tryptophan are very small. If only these data were available one might be justified in concluding that 1.8% of the D isomer would promote maximum growth in the chick. When consideration is given to the second experiment with the suggested value of 2.6% of the D isomer for maximum growth, it seems safer to use the 2.1% level as the tentative minimum requirement on this diet. Fitting a straight line to these data revealed a nonsignificant slope and a curve did not reduce the error mean square so that, statistically, it was impossible to establish a difference between any of these values.

The growth on the 2.4 and 2.7% levels of D-tryptophan, while below that of the 2.1%, does not indicate a toxic effect of D-tryptophan. This lack of toxicity of the D isomer at high levels is in agreement with work by Van Pilsum and Berg ('50) and in disagreement with a report by Albanese and Irby ('43).

It is realized that the optimum level of D-tryptophan may in reality be less than the 2.1% level. To establish this point with more certainty would require a more elaborate experiment. It seemed reasonable, however, using a larger number

of chicks, to establish that 2.1% of D-tryptophan would cause chicks to grow as well as those receiving an adequate amount (0.2%) of the L isomer. Therefore in the 4th experiment D-tryptophan was present at 2.1% and L-tryptophan was provided in excess of the 0.15% required, by the use of 0.4% of the DL-mixture. These data leave little doubt that with this diet the D isomer, if provided in sufficient quantity, can be used by the chick for the synthesis of body tissue and this synthesis occurs at a rate comparable to that of chicks fed the L isomer.

Parenteral administration of tryptophan. The finding that orally administered D-tryptophan was poorly utilized by the chick, suggested the feasibility of investigating the reason for this inefficiency. Possible reasons for this inefficient utilization are: (1) The body tissue is unable to freely metabolize the D isomer or, more precisely, is unable to efficiently convert the D form to the L form, or (2) the animal lacks the ability to absorb this amino acid efficiently, or (3) the animal body cannot use the D isomer and the high requirement reflects the inefficient conversion of the D isomer by the intestinal microflora.

While the literature contains evidence that D-lysine is not attacked by D-amino acid oxidase (Klein and Handler, '41; Ratner et al., '43; Neuberger and Sanger, '44), such is not the case with D-tryptophan. This suggested that inefficient conversion within the body was not likely to be a major factor. In contrast to this, difficulty in the absorption of the D-isomer has been reported for many amino acids.

Early work had indicated no difference in the absorption of the various isomers (Berg and Baugess, '32; Chase and Lewis, '34; Featherstone and Berg, '42). More recent studies by Gibson and Wiseman ('51) question the validity of this viewpoint. These workers reported the existence of a specific mechanism for the active absorption of the L-amino acids. Use was made of ligatured loops of rat's intestine *in vivo* and 13 amino acids were studied. In addition Wiseman ('51) found by *in vitro* technique, in which isolated rat intestine was used, that the L-isomers of several amino acids were transferred

against a concentration gradient while the corresponding D isomers were not. In 1953 Agar et al. reported similar findings with the L and D isomers of phenylalanine and histidine.

While these investigations suggested the possibility of somewhat similar studies in the present work, the parenteral administration of the D isomer seemed to offer additional advantages. It was felt that if the intestine were by-passed an answer to (1) and the first part of (3) could be obtained as well as some information as to the absorption of the D isomer.

TABLE 3

Weight and gain of individual chicks receiving 70 mg of L-tryptophan daily by injection every three hours — Experiment 5

SUPPLEMENT TO BASAL	TIME IN HOURS				
	0	24	48	72	96
	Weight				
	gm	*gm*	*gm*	*gm*	*gm*
L-tryptophan	175	185	195	201	202 (27)[1]
L-tryptophan	182	202	208	212	230 (48)
L-tryptophan	190	208	217	225	238 (48)
L-tryptophan	188	201	211	216	223 (35)
None	179	180	178	177	179 (0)
None	197	197	198	196	194 (−3)
None	173	174	171	170	171 (−2)

[1] Figures within parentheses represent gain or loss in weight from zero time.

Relatively litle work has been done with regard to the injection of individual amino acids. Jackson ('27), Alcock ('34) and Yeshoda and Damodaran ('47) reported that injected tryptophan failed to support growth. On the other hand du Vigneaud et al. ('32, '35 '36) found that rats gained weight if given tryptophan by injection. These papers provided little information as to techniques and it was thus necessary to carry out several preliminary experiments before adopting the procedure described earlier.

The initial work was carried out by injecting the L isomer. Typical results with individual chicks are given in table 3. In the preliminary work the level of 70 mg per day was used be-

cause it represents the calculated amount of L-tryptophan required daily by chicks of the size used. The results showed clearly that the subcutaneous injection of L-tryptophan promoted growth in the chick.

Since the ultimate aim of this study was to compare the utilization of the injected L and D isomers it was necessary to

TABLE 4

Growth of chicks injected every three hours with D- *or* L-*tryptophan*

EXPERIMENT NO.	GROUP	TREATMENT	TIME IN HOURS				
			0	24	48	72	96
			Average weight				
			gm	*gm*	*gm*	*gm*	*gm*
6 (10)[1]	1	None	149	146	147	146	
	2	70 mg L-tryptophan/chick/day	148	161	169	176	
	3	70 mg D-tryptophan/chick/day	153	157	162	165	
7 (6)	1	None	170		162		158
	2	35 mg L-tryptophan/chick/day	170		182		192
	3	70 mg L-tryptophan/chick/day	171		189		209
	4	105 mg L-tryptophan/chick/day	172		193		213
	5	140 mg L-tryptophan/chick/day	171		190		210
	6	175 mg L-tryptophan/chick/day	170		188		212
8 (6)	1	140 mg D-tryptophan/chick/day	211	219	219	223	
	2	175 mg D-tryptophan/chick/day	210	216	222	232	
	3	210 mg D-tryptophan/chick/day	207	219	226	236	
	4	245 mg D-tryptophan/chick/day	211	223	234	245	
	5	280 mg D-tryptophan/chick/day	211	221	230	236	
	6	91 mg L-tryptophan/chick/day	210	223	233	246	

[1] Figures within parentheses indicate the number of chicks per experimental group.

establish the requirement for both isomers for optimum growth when administered by the injection technique. First, however, it seemed desirable to establish that the D isomer was utilized by the chick when given parenterally. Accordingly an experiment was performed in which the L and D isomers were provided at 70 mg per day. Table 4 (experiment 6) contains the relevant data. A highly significant growth response resulted from the injection of the D isomer.

The results make it necessary to re-evaluate much of the previously reported work. Contrary to the conclusions drawn by Anderson et al. ('50) and Almquist ('52) the tissues of the chick can, apparently, metabolize and use D-tryptophan for growth.

A further point worthy of note is that this growth resulted on a level of D-tryptophan equivalent to the level used for L-tryptophan. In contrast, when a level of D-tryptophan is fed which is equivalent to the fed L-tryptophan requirement, no growth results. This would suggest that inefficient conversion within the body is not the major cause for the extremely high requirement for D-tryptophan when given *per os*. It also suggests that, while the microflora may be necessary for the utilization of the orally administered D isomer, this is not because of an inability on the part of the tissues to convert the D isomer. In view of these results the very high requirement for orally administered D-tryptophan appears to be due to the fact that this form is absorbed very inefficiently, if at all.

The utilization of parenterally administered D-tryptophan by the chick having been established, it was possible to determine the requirements for the L and D isomers when given by injection. For the determination of the injected L-tryptophan requirement, graded levels of the L isomer were given ranging from 35 mg per day to 175 mg per day. The results of experiment 7 are given in table 4 and figure 1.

A line fitted to the first three arrays had a highly significant slope while the slope of the line fitted to the last two arrays was non-significant. Calculation of the point of intercept revealed an injected L-tryptophan requirement of approximately 91 mg per day. On the basis of the food consumption for the three highest levels it is calculated that the amount of L-tryptophan required per day would be 46 mg. Hence it appears that the procedure used doubles the chick's requirement for this amino acid.

A similar procedure was followed in the 8th experiment in estimating the requirement for D-tryptophan by the injection technique. The levels ranged from 140 to 280 mg of D-tryp-

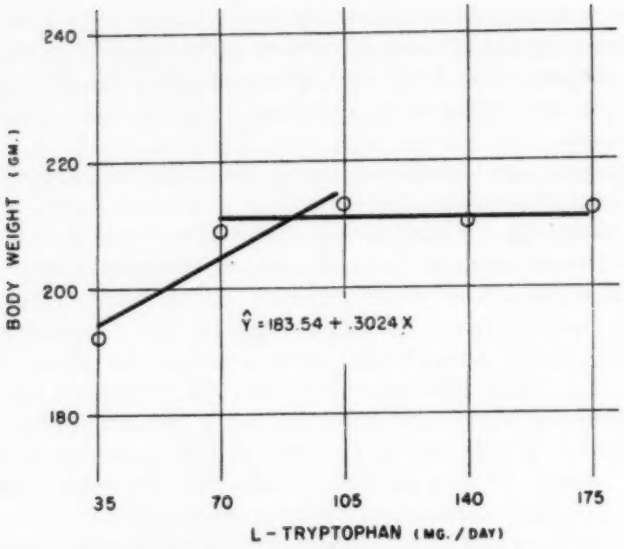

Fig. 1 Requirement for injected L-tryptophan.

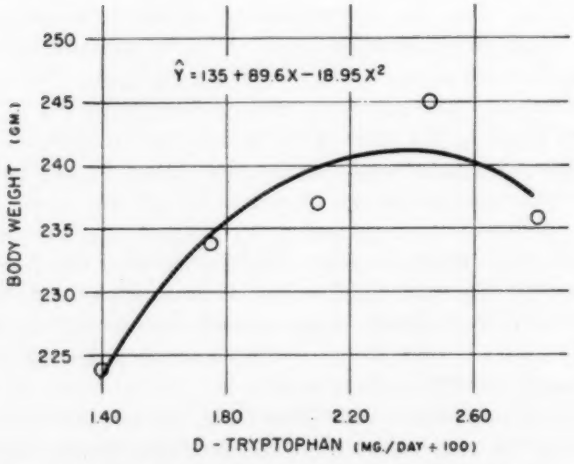

Fig. 2 Requirement for injected D-tryptophan.

tophan per day with increments of 35 mg per day. In addition group 6 was injected with L-tryptophan at the level previously determined as the requirement.

Probably the first point of interest is that growth obtained by the use of the D isomer was equivalent to that noted when the L isomer was used. This demonstrates that the chick's tissues can convert enough D-tryptophan to meet at least 80% of its requirement for this amino acid because, as pointed out earlier, the basal diet contained approximately 0.036% of L-tryptophan which is equivalent to approximately 20% of the chick's requirement for this amino acid.

A quadratic equation (fig. 2) describing the data indicated a requirement for injected D-tryptophan of approximately 236 mg per day. A curve was used in this case because of the decreased growth on the highest level of injected D-tryptophan.

In addition to growth experiments a nitrogen-balance study was conducted and although the results are not presented the data showed clearly that the injection of L- or D-tryptophan caused a highly significant increase in nitrogen retention.

DISCUSSION

The data of experiments 2 to 4 indicate that the unsupplemented diet provided tryptophan only slightly in excess of the maintenance requirement of chicks of this size. In order to obtain optimum growth on this diet 0.15% of L-tryptophan must be added (Morrison, '55). To obtain equivalent growth with the D isomer 2.1% is required which is 14 times the L-tryptophan requirement. This points up a weakness inherent in the use of the DL mixture for establishing D isomer utilization. In this case, if one were comparing 0.14% of L-tryptophan to 0.28% of DL-tryptophan, it would be the equivalent of comparing 0.14% of the L isomer to 0.149% of the L isomer. Such a small difference cannot be detected by the usual experimental technique. Moreover, use of the racemic form makes it difficult to estimate the ability of the animal to convert the D isomer beyond 50% of its requirement for that

amino acid. The other 50% is present as the L form since the DL mixture provides essentially equal amounts of the two isomers. Thus expressing the utilization of the D isomer as a percentage is open to question unless the conditions under which the utilization was determined are described.

As a result of this work it can be stated that when the diet contains approximately 0.036% of tryptophan (18% of the L-tryptophan requirement) as the L isomer, the D form will cause optimum growth if provided at 14 times the supplemental L-tryptophan requirement. On this basis the activity of the D isomer is only 7% of that of the L form. This study strongly suggests that in a diet free of the L isomer, the D form could be used as the sole source of this amino acid because, in this diet, it is replacing 0.15% of L-tryptophan. On a diet in which cerelose or dextrin replace corn, the total L-tryptophan requirement is approximately 0.15% (Fisher et al., '55; Griminger, '55). Nevertheless it cannot be stated that the D isomer can meet the entire tryptophan requirement until a diet, devoid of tryptophan and giving satisfactory growth, is used. Work in this laboratory has indicated that in a diet containing dextrin as the carbohydrate source, and thus free of tryptophan, the D isomer does support good growth. Sufficient work has not been done, however, to establish that, on such a diet, the D isomer would support growth equivalent to that promoted by the L isomer. The fact that the D isomer is utilized, even though none of the L isomer is present, is in contrast to work by Celander and Berg ('53a) who reported that, in the mouse, the presence of the L isomers was necesary in order for the D isomers of tryptophan and histidine to be utilized.

These results provide support for those who have previously reported utilization of orally administered D-tryptophan by the chick. The disagreement between these results and the 17 to 40% suggested by Wilkening and Schweigert ('47) may reflect the larger amount of the D isomer being converted in the present study.

As pointed out earlier, the approach taken to this problem precluded associating the entire cause of the inefficient utilization of D-tryptophan with ineffective absorption. It does seem most probable, however, that this step is the primary impediment in the utilization of this D isomer. That it is not due to the lack of ability on the part of the tissues to convert the D isomer to the L form appears evident. This is assuming, of course, that the D isomer is converted to the L form before being built into body tissue, as has been shown for D-histidine by Conrad and Berg ('37) and as suggested by the work of Walser et al. ('50).

With this consideration in mind this study provides an answer to (1) and the first part of (3) listed earlier, namely, that the body tissues are able to utilize D-tryptophan and can do so efficiently.

This is very clearly seen when one compares the requirements of D- and L-tryptophan when fed and when administered parenterally. When these isomers are fed the ratio is approximately 14:1 and when they are injected the ratio is decreased to approximately 2.5:1. On the basis of the food consumed and the *per os* requirement of 2.1%, those chicks receiving the D isomer by injection should have required 420 mg D-tryptophan per day. Instead of this, however, the requirement was approximately 236 mg per day. On the same basis the L-tryptophan requirement should have been 48 mg per day but was shown to be 91 mg per day. Thus the injection technique decreased the chick's total requirement for the D isomer and increased its total requirement for the L isomer. These facts seem to be most readily explainable on the basis of a block in the absorption of the D isomer. This is in agreement with work in the rat by Gibson and Wiseman ('51), Wiseman ('51), Agar et al. ('53).

If absorption is the answer to this problem it is still impossible, on the basis of these data, to say whether the chick cannot absorb the D isomer at all or can absorb it only inefficiently. If the former is true, the growth resulting from high levels of orally administered D-tryptophan must be due

to conversion of the D-isomer to the L form within the intestinal tract, probably by the microflora. If the latter supposition is correct the microflora may or may not play a role in its utilization. Work by Anderson et al. ('50) suggests that the microflora is indeed important in this regard. If the microflora is acting in this way, however, it is only because the D-tryptophan is not reaching the tissues in sufficient quantity and not due to an inability on the part of the tissues to utilize this isomer. The nonessentiality of the microflora for utilization of D-histidine by the rat has been shown by Celander and Berg ('53b).

The greater requirement for injected D-tryptophan over the injected L-tryptophan undoubtedly reflects the loss of the former during conversion. As cited by Krebs ('51) the most active site for D-amino acid oxidase is in the kidney. It is probable that some loss of the keto acid occurs during its transportation from the kidney to the liver.

The injected D-tryptophan gave no evidence of being toxic, except for the highest level, despite the relatively large amounts given. Abderhalden and Tetzner ('35) found that intramuscularly injected D-alanine caused a severe reaction in pigeons. Albanese and Irby ('43) felt their work showed that the D isomers of certain amino acids were toxic. Several others workers have failed to support the conclusions of Albanese and Irby and Van Pilsum and Berg ('50) found the L isomer of methionine to be the more toxic.

Wretlind ('52) has pointed out that when the utilization of a D-amino acid is studied, the presence of other amino acids in the D form should be considered. The diet used in this present study contained 0.30% of DL-methionine but how important this was in this instance cannot be stated.

SUMMARY

The utilization of orally administered D-tryptophan was studied using growth as the criterion. A diet containing approximately 0.036% of tryptophan was supplemented with

graded levels of the D isomer. The results suggest that D-tryptophan, if added to the diet of the young chick in sufficient quantity, will promote growth comparable to that obtained by the use of the L isomer. The approximate supplemental requirements on this diet for orally administered L- and D-tryptophan are 0.15 and 2.1% respectively. The utilization of the D isomer as found in this study indicates a supplemental requirement for the DL mixture of approximately 0.28% of the diet.

Chicks approximately 17 days of age receiving a tryptophan-deficient diet were provided with L- or D-tryptophan by means of subcutaneous injections. Growth and an increase in nitrogen retention resulted when either isomer was given. The approximate requirements for L- and D-tryptophan when administered in this way are 91 and 236 mg/chick/day respectively. This work shows that the chick can utilize the D isomer without the assistance of the microflora and suggests that inefficient absorption is the cause of the very high requirement for orally administered D-tryptophan.

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FOOD INTAKE AND ESTROGENIC HORMONE EFFECTS ON SERUM AND TISSUE CHOLESTEROL [1,2]

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(Received for publication March 5, 1956)

Cholesterol-fed male rats have shown greater variations in liver cholesterol with different levels of protein intake than have females (Okey and Lyman, '54, '56). In young rats, the sex differences became apparent at the time when males showed the heightened food consumption and growth rate associated with the attainment of puberty. Moreover, they were most marked when the diets contained barely enough good protein to support optimal growth if the total food (and hence calorie) consumption was high. An investigation seemed indicated, to test whether the sex difference was the result of a specific hormone effect or only a corollary of the difference in food intake and growth rate of males and females. Human males are known to have a higher incidence of diseases associated with abnormal tissue cholesterol deposition, whereas human females (as well as female rats) tend to have higher serum cholesterols than males of the same age and diet groups (Gillum et al., '55). It was therefore also considered desirable to find out something about the relationship between circulating and stored cholesterol.

[1] Supported in part by grant-in-aid H-1013, National Heart Institute, U. S. Department of Health, Education and Welfare and by funds appropriated under the Research and Marketing Act of 1946 as part of Western Regional Experiment Station Project W-4.

[2] Presented at the Atlantic City Meeting of the Federation of Societies for Experimental Biology, April 1956.

EXPERIMENTAL

Two studies were carried out. For the first, 6 groups of 10 150-gm male rats each were studied. Three were placed on the 15% casein-egg albumin diet with 1% cholesterol, previously described [3] (Okey and Lyman, '56), and three on the corresponding 30% protein diet. Two of the groups fed each diet were castrated, and the third served as intact controls. One castrate group on each diet was given 0.05 mg estradiol benzoate in sesame oil, by subcutaneous injection, three times weekly; the other was given sesame oil only. All were fed ad libitum. Weights and food intakes were recorded. Hormone dosage began three days after castration. Three weeks later the rats were autopsied, and the livers and adrenals were prepared for analyses as previously described (Okey and Lyman, '54).

The second study was undertaken because the marked diminution of food intake and growth rate produced by the hormone indicated a need for more data concerning the effect of the quantity of food ingested. For this second study, 110 additional male rats were used. Two-thirds of them were castrated at 150 gm. They were grouped as intact controls, castrate controls, and hormone-dosed castrates. Half of each group was fed the 15% protein diet, half the 30%. Rats of the hormone-dosed castrate subgroup fed 30% protein were used as pacemakers. Two-thirds of the rats from the two intact control groups, the two castrate control groups, and the hormone-dosed castrate group fed 15% protein were given only the mean daily amount of food consumed by the rats of the pacemaker group. In each diet group, the remaining third of the rats was fed ad libitum. The hormone dosage was one-third lower than that given in the first study. After three

[3] The percentage composition of the diets was as follows: (a) *"15% protein"*: egg albumin, 10; vitamin-free casein, 5; fat (Primex), 13.5; sucrose, 64.5; salts, 4; fat soluble vitamin mix, 1; "B" vitamin mix, 1; cholesterol, 1; (b) *"30% protein"*: egg albumin, 25; vitamin-free casein, 5; fat (Primex), 13.5; sucrose, 49.5; salts, 4; fat-soluble vitamin mix, 1; "B" vitamin mix, 1; cholesterol, 1.

weeks' dosage, the rats were sacrificed. Procedures for autopsy and tissue analyses were those previously used, except that blood was collected at decapitation. Serum and tissue phospholipids were determined by the method of Fiske and Subbarow ('25).

RESULTS AND DISCUSSION

In the two studies, there was little difference between the mean food intakes and growth rates for the corresponding groups of ad libitum-fed rats. Growth data for those of the second study, as shown in figure 1 may therefore be considered typical for all the ad libitum-fed animals.

Gains of control rats were slightly greater with the 15% protein diet than with the 30%. Castrates ate a little less and grew somewhat less well than did intact controls. But

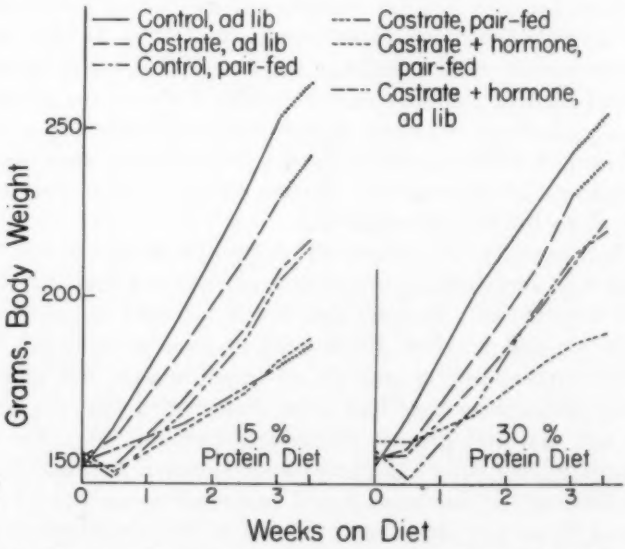

Fig. 1 Mean growth curves for the rat groups of the second study. Lines broken at the ends represent animals fed ad libitum.

the only really significant differences were shown by the hormone-dosed animals. These ate from 75 to 85% as much food as the undosed castrates; but the corresponding weight gains ranged from 33 to 40% and 31 to 45%, respectively, of those of the ad libitum-fed rats on the 15 and 30% protein diets (fig. 1). The decrease in hormone dosage in the second study had no significant effect either on the food intake or on weight gains. Pair-fed intact and castrate rats gained approximately twice as much as their hormone-dosed littermates with the same food intakes.

The *lipid* and *cholesterol data* for the matched ad libitum and pair-fed rats of study 2 are given in table 1. While the detailed data for the first study are omitted, the fact that the proportionate differences in lipids and cholesterol between the corresponding ad libitum groups in study 1 were much the same as those given in the table deserves mention. This is so because two-thirds of the rats used in study 2 were pair-fed — i.e., each ad libitum subgroup consisted of 6 to 8 rats, while the pair-fed groups were made up of 12 to 14 rats each. The consistency of the findings for the additional subgroups of 10 ad libitum-fed rats each in study 1 therefore assumes some significance. (Actual figures for the two studies were not averaged, both because of the difference in hormone dosage and because, inadvertently, different batches of fat were used in the diets for the two studies.)

In both studies, the intact, ad libitum-fed males given 30% protein had significantly lower liver cholesterol than did those fed 15% protein. Castrates fed the two levels of protein ad libitum showed smaller differences in liver cholesterol than did the corresponding groups of intact males, but slightly greater differences than had been observed in females of the same age and diet groups (Okey and Lyman, '56). The differences in liver lipid and cholesterol, between the two groups of ad libitum-fed, hormone-dosed castrates given 15 and 30% protein, were not significant. The low liver cholesterols in the hormone-dosed rats pair-fed the 15% protein diet may possibly have been associated with their disproportionately

TABLE 1

Serum and liver lipids
(Mean Values)

DIET GROUP	LIVER						SERUM		
	Wt.	Total lipid	Cholesterol		Phospho-lipid	Cholesterol to phospholipid ratio	Choles-terol	Phospho-lipid	Cholesterol to phospholipid ratio
	gm	*% moist wt.*	*% moist wt.*	*mg/liver*	*% moist wt.*		*mg %*	*mg %*	
					15% protein				
Control									
Ad libitum	11.4	15.8	4.21 ± 0.33 [1]	422 ± 73	3.55 ± 0.13	1.14 ± 0.09	98 ± 12	146 ± 16	0.67 ± 0.13
Pair-fed	9.8	13.2	2.76 ± 0.15	272 ± 21	3.74 ± 0.22	0.76 ± 0.25	100 ± 12	129 ± 10	0.73 ± 0.06
Castrate									
Ad libitum	11.0	13.4	3.33 ± 0.34	356 ± 34	3.38 ± 0.18	0.98 ± 0.10	103 ± 12	142 ± 14	0.71 ± 0.04
Pair-fed	8.8	11.0	2.63 ± 0.37	233 ± 30	3.69 ± 0.13	0.71 ± 0.10	117 ± 8	132 ± 5	0.89 ± 0.15
Castrate + hormone									
Ad libitum	7.9	11.5	4.29 ± 0.31	350 ± 31	3.43 ± 0.24	1.28 ± 0.11	284 ± 30	234 ± 15	1.22 ± 0.11
Pair-fed	8.2	9.5	3.20 ± 0.31	260 ± 30	3.43 ± 0.12	0.87 ± 0.10	352 ± 37	235 ± 8	1.49 ± 0.14
					30% protein				
Control									
Ad libitum	10.3	10.5	2.97 ± 0.40	296 ± 33	3.96 ± 0.19	0.74 ± 0.09	102 ± 9	144 ± 17	0.72 ± 0.03
Pair-fed	8.9	9.7	2.62 ± 0.21	223 ± 24	4.21 ± 0.22	0.64 ± 0.05	138 ± 13	154 ± 10	0.89 ± 0.07
Castrate									
Ad libitum	9.3	10.3	2.98 ± 0.47	270 ± 38	3.38 ± 0.18	0.77 ± 0.11	120 ± 10	166 ± 7	0.77 ± 0.03
Pair-fed	8.8	10.3	2.67 ± 0.33	228 ± 22	4.09 ± 0.12	0.65 ± 0.07	163 ± 14	148 ± 8	1.09 ± 0.09
Castrate + hormone									
Pacemakers	8.8	10.6	4.12 ± 0.21	362 ± 20	3.36 ± 0.11	1.24 ± 0.07	275 ± 22	235 ± 11	1.17 ± 0.08

[1] Standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

large initial weight loss after castration. However, older female rats have been observed in this laboratory to show some tendency toward increases in liver cholesterol on long continued feeding of high protein diets.

Most interesting, however, was the contrast between the serum and liver lipid values for the ad libitum-fed and the restricted rats of the corresponding groups. Differences in liver cholesterol with differences in the protein content of the diet were almost eliminated in the rats with restricted food intake. But *serum* cholesterols were, in each case, higher for the restricted than for the ad libitum-fed rats. Values for castrates were approximately as much higher than serum cholesterols for controls as values for females have been observed to be higher than those for males. Rats fed 30% protein showed a greater increase in serum cholesterol with restriction of food intake than did those fed 15% protein.

On the assumption that mobility of circulating cholesterol may possibly be related to phospholipid content of serum, the latter was determined. Serum phospholipids were found to be decreased slightly by restriction of food intake. This meant that, for the 30% protein groups, differences in cholesterol-phospholipid ratios produced by dietary restriction were statistically significant ($p < 0.05$ and $p < 0.02$ for the controls and castrates, respectively) in spite of the fact that, as noted earlier, the comparison was made on the basis of data for the comparatively small groups fed ad libitum in the second study.

Concentration of *liver phospholipid* was higher in the *undosed* rats receiving 30% protein than in those receiving 15%. Restriction of food intake seemed to increase the concentration slightly in the intact control and in the castrate rats fed both diets. The net result was a lowering of the cholesterol/phospholipid ratios in the livers of the undosed, restricted rats. The extent of this lowering was slightly greater in the rats fed 15% protein, where liver cholesterols were high in the ad libitum groups. In all of the *hormone-dosed* rats, however, liver phospholipid concentrations were low and serum phos-

pholipid increases were not so great as the increases in serum cholesterol. The cholesterol-phospholipid ratios with estradiol benzoate dosage were, therefore, high and much alike in both serum and liver.

It seems logical, therefore, to consider the hypothesis that the effect of the hormones and that of dietary restriction may be independent, but that, under some conditions, they may augment each other.

In the intact, pair-fed male *controls,* presumably, the growth promoting and testicular hormones were active. The lowering of liver cholesterol, with its concomitant increase in serum cholesterol, could therefore be attributed to the decreased food intake, possibly augmented by some increase in the efficiency of cholesterol absorption.

In the *castrates,* testicular hormones were absent, but adrenal and pituitary hormones were functioning presumably uninhibited by the sex hormones. Nevertheless, the ad libitum-fed castrates had liver cholesterols which were about as much lower than those of the intact controls as were their total food intakes, a finding which did not indicate any specific effect of the lack of testicular hormone on liver cholesterol storage.

The increases in *serum cholesterol* with dietary restriction were greater in castrates than in intact animals, a result which might be explained by the absence of a male-hormone effect in lowering circulating cholesterol. The increases in serum cholesterol in the estrogen-dosed castrates were, however, far greater than those in the undosed castrates with the same food intake (75 to 120%). At the same time, *liver* cholesterols in the hormone-dosed rats remained higher than the food intakes and growth rates of these animals might lead one to expect. The hormone seemed therefore to have a specific effect. It was not, however, clear whether it acted by mobilization of dietary cholesterol, presumably for hormone production; by stimulation of cholesterol synthesis; or by producing a decrease in turnover rate which overbalanced the effect of decreased food intake on liver cholesterol accumulation.

As one means of testing for evidence of increased mobilization for hormone secretion, *adrenal cholesterols* were determined (table 2). Adrenal cholesterol concentrations were considerably increased by dietary restriction. This change reflected differences of only 0.2 to 0.5 mg cholesterol per

TABLE 2

Adrenal cholesterol

(Means and standard errors)

GROUP AND DIET	NO. RATS	WT. OF ADRENALS	CHOLESTEROL	
		mg	*% moist wt.*	*mg*
		15% protein		
Control				
Ad libitum	6	37.6	3.48 ± 0.36 [1]	1.33 ± 0.19
Pair-fed	12	33.3	4.75 ± 0.36	1.59 ± 0.13
Castrate				
Ad libitum	7	38.5	3.51 ± 0.37	1.39 ± 0.22
Pair-fed	13	36.5	5.21 ± 0.29	1.91 ± 0.12
Castrate + hormone				
Ad libitum	7	50.8	4.79 ± 0.26	2.41 ± 0.11
Pair-fed	12	44.6	5.19 ± 0.27	2.32 ± 0.13
		30% protein		
Control				
Ad libitum	6	39.9	3.81 ± 0.51	1.51 ± 0.17
Pair-fed	12	34.2	4.64 ± 0.31	1.59 ± 0.11
Castrate				
Ad libitum	7	42.1	3.31 ± 0.26	1.43 ± 0.21
Pair-fed	14	37.9	4.46 ± 0.32	1.68 ± 0.12
Castrate + hormone				
Pacemakers	13	49.5	4.34 ± 0.14	2.13 ± 0.14

[1] Standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

adrenal because of the slightly smaller size of the adrenals in the restricted rats. However, the size of the adrenals was significantly greater (8 to 14 mg) in the hormone-dosed animals, as were the percentage and amounts of cholesterol. Whether or not this indicates that administration of estrogenic hormone stimulated production of cortical hormones from cholesterol, the differences were somewhat larger than

those usually found between adrenals of male and female rats.

The data as a whole indicate that both restriction of food intake and dosage with estrogenic hormone are capable of altering cholesterol transportation and storage. In both cases the primary effect seems to be an increase in the circulating cholesterol. With restriction of total food intake, this increase is greater when the diet is high in protein. With estrogenic hormone, serum cholesterols were so high that relation to diet became doubtful.

That the increase in circulating cholesterol can take place at the same time that liver cholesterol stores are depleted adds one more reason for doubting the value of serum cholesterol determinations as an index of tissue cholesterol retention. The fact that serum cholesterols were consistently increased by restriction of food intake, which meant restriction of cholesterol intake as well, also raises some questions of interpretation of data.

McMillan et al. ('54) have reported increases in serum cholesterol with lowering of food intake in rabbits dosed with set amounts of cholesterol. They found no increase in the incidence or severity of atherosclerosis in their underfed animals. Rabbits are rated as more likely to show tissue cholesterol deposits in response to cholesterol feeding than are human beings, but rats are especially resistant to development of cholesterol lesions. It should be interesting to follow, at frequent intervals, the circulating cholesterol level in human atherosclerosis patients who are consuming high protein diets as a means of controlling weight. Also, obviously, correlation of autopsy data with dietary histories is indicated.

SUMMARY

An ad libitum and a pair-feeding study are reported for castrated male rats fed 1% cholesterol with 15 and with 30% protein. In each case one castrate group was treated with estradiol benzoate.

Food intakes of the hormone-dosed castrates were reduced one third to one fourth as compared with ad libitum controls, intact and castrate, respectively. However, when undosed castrates were pair-fed to those dosed with hormone, they gained approximately twice as much.

The ad libitum intact controls showed the usual higher liver cholesterol with the 15 than with the 30% protein diet. Variation in liver cholesterol with protein content of the diet was less in castrates than in controls.

In the undosed rats restricted as to food intake, *serum cholesterols* were higher and *liver cholesterols* lower than in the ad libitum-fed rats. The increase in serum cholesterol with restriction of food intake was greater in the rats fed 30% protein.

Serum cholesterols were very high and adrenal cholesterols were increased in the hormone-dosed rats, but *liver cholesterols* for both diet groups were within the ranges of those of the intact controls fed 15% protein. Data are discussed in terms of relationship of food and cholesterol intake to serum and liver cholesterol.

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THE RELATION OF DIETARY SUPPLEMENTS AND TISSUE METABOLITES TO GLYCINE TOXICITY IN THE CHICK[1]

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(Received for publication April 6, 1956)

INTRODUCTION

Glycine toxicity in the chick can be prevented by the administration of high levels of folacin (Naber, Snell and Cravens, '52; Machlin, Lankenau, Denton and Bird, '52), but it is not known why glycine is toxic nor how folacin enables the chick to metabolize excess glycine successfully. Folacin is directly or indirectly concerned with several metabolic reactions of glycine, including conversion to serine (Elwyn and Sprinson, '50; Broquist, '49), the synthesis of purine bases and hence of uric acid (Shemin and Rittenberg, '47; Buchanan, Sonne and Delluva, '48) and the incorporation of formate into serine and heme (Plaut, Betheil and Lardy, '50). Pathways not thought to be mediated by folacin involve the conversion of glycine to creatine (Bloch and Schoenheimer, '41), glyoxylic acid, or oxalic acid (Ratner, Nocito and Green, '44). Folacin-deficient chicks might therefore have a diminished capacity for the metabolism of glycine through normal channels, and hence toxicity could result either from the presence in the body of excess glycine itself, or of a metabolite arising from reactions

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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that take precedence when folacin is limiting. In the present study measurements have been made of likely metabolites in the tissues of chicks fed excess glycine, and attempts made to alter the severity of the toxicity with appropriate nutrients.

EXPERIMENTAL

Day-old New Hampshire × Single Comb White Leghorn crossbred chicks of both sexes obtained from dams fed ration B-1 of Robblee, Nichol, Cravens, Elvehjem and Halpin ('48) were reared in electrically heated batteries with raised screen floors. Feed and water were supplied ad libitum. Ten to 25 chicks were allotted at random to each of the experimental groups. The composition of the basal ration was sucrose 600, hot-alcohol extracted casein 180, gelatin 100, DL-methionine 3, soybean oil 50, fish oil (1500-A, 300-D) 5, salts V (Briggs, Luckey, Elvehjem and Hart, '43) 60 and choline chloride 2. Vitamins were added as follows in milligrams per kilogram of ration: thiamine·HCl 6.0, riboflavin 6.0, niacin 100.00, calcium pantothenate 20.0, pyridoxine·Cl 4.0, biotin 0.2, 2-methyl-1,4-naphthoquinone 0.5, alpha tocopherol acetate 3.0, para aminobenzoic acid 100.0 and meso inositol 1000.0. Vitamin B_{12} was supplied in crude form to provide 50 μg/kg of ration. This ration did not contain any added folacin. All supplements were included at the expense of sucrose.

Blood glucose determinations were made with the anthrone reagent as described by Durham, Bloom, Lewis and Mandel ('50). Liver glycogen analyses were made by the direct method of Seifter, Dayton, Novic and Muntwyler ('50). Uric acid was measured according to the method of Brown ('45). Blood glycine levels were measured by the method of Alexander, Landwehr and Seligman ('45). Para-hydroxyphenyl compounds were determined in blood serum as described by Seydl, Thiele and Boguth ('50) with para-hydroxyphenyl pyruvic acid as a standard. This latter was synthesized and purified according to the procedure described by Herbst and Shemin for phenylpyruvic acid ('43). Glyoxylic acid was prepared by the method of Benedict ('09).

Serum calcium determinations were made by the Clark-Collip modification of the Kramer-Tisdall method published by Hawk, Oser and Summerson ('49). Microbiological assays for folacin were made on the liver, kidney, pancreas and spleen of chicks as described by Sunde, Cravens, Bruins, Elvehjem and Halpin ('50), except that spleen and pancreas samples were autolysed at pH 7.0 to secure maximum liberation of bound folacin. In one experiment the toxicity of certain compounds was tested by depositing aqueous solutions directly into the crop of the chick with the aid of a syringe fitted with a small plastic tube.

RESULTS

As in previous studies the addition of 5% of glycine to a semisynthetic low-folacin diet depressed growth and produced symptoms of toxicity such as nervousness, tremors, protruding eyes, and occasional paralysis, whereas in the presence of folacin there were no symptoms nor was growth affected by glycine (table 1). The most significant biochemical changes observed were in liver glycogen, which was depressed by glycine and increased by folacin. Blood glucose, uric acid, calcium, and *p*-hydroxyphenyl compounds were unaffected by either glycine or folacin. Blood glycine was increased about three-fold when glycine was fed, but the addition of folacin to the diet did not reduce these levels (table 1). Conversely, the addition of a toxic level of glycine to a crude practical ration failed to depress the folacin content of the tissues, and in the liver and pancreas, folacin was actually somewhat higher in the birds fed glycine (table 2).

The specificity of glycine toxicity was demonstrated by the failure of serine to depress growth or to cause any symptoms of toxicity (table 3; also Naber et al., '52) while the growth-depressing effects of alanine and tyrosine were not corrected by folacin (table 3). Furthermore, the birds fed these latter acids did not exhibit typical symptoms of glycine toxicity.

In other trials a dietary supplement of the creatine precursors arginine or methionine or both, largely prevented

TABLE 1

The effect of folacin and glycine on blood and organ levels of various components

GROUP NO.	SUPPLEMENT TO BASAL RATION		AV. WEIGHT AT 2 WKS. (ALL EXPS.)	AVERAGE VALUES [1] AFTER 2 WEEKS							
				Blood	Glucose	Liver glycogen	Blood uric acid	Blood glycine	Blood p-hydroxyphenyl compounds		Serum calcium
	Folacin	Glycine		Exp. C-7	Exp. C-8	Exp. C-15	Exp. C-9	Exp. C-12	Exp. C-10	Exp. C-11	Exp. C-16
	mg/kg	%	*gm*	*mg %*	*mg %*	%	*mg %*	*mg %*	*mg %*	*mg %*	*mg %*
1	..	..	82	200	241	2.7 [2]	8.3	11.1 [2]	14	15	10.2
2	..	5	62 (Severe toxicity symptoms)	206	210	1.3	8.6	28.1	14	16	10.4
3	10	..	109	237	208	5.6	8.1	11.9	18	17	10.4
4	10	5	110	222	201	3.2	9.2	33.9	14	16	10.3

[1] Each value represents the average of 4 to 10 determinations.

[2] Analysis of variance indicates that statistically significant differences at the 1% level exist, with liver glycogen, between group 1 and groups 2 and 3; with blood glycine, between group 1 and groups 2 and 4.

the toxicity symptoms of glycine, but did not restore the growth of the glycine-fed chicks.

Two per cent of sodium formate caused a small increase in growth when added to the folacin-deficient basal ration in each of three trials (table 4), but showed no beneficial effect

TABLE 2

Folacin content of organs from chicks fed glycine

SUPPLEMENT TO PRACTICAL TYPE RATION (Exp. C-5)	AV. WEIGHT (AFTER 10 DAYS)	AVERAGE FOLACIN CONTENT [1] (μG/GM FRESH TISSUE)			
		Liver	Kidney	Pancreas	Spleen
	gm				
None	357	7.1 [2]	1.9	0.23 [2]	0.12
10% Glycine	302	12.3 [2]	1.9	0.39 [2]	0.09

[1] Each value represents the average of 15 determinations.

[2] Analysis of variance indicates differences statistically significant at 1% level.

TABLE 3

Effect of glycine, DL-*serine,* DL-*alanine and* L-*tyrosine on chick growth*

SUPPLEMENT TO BASAL RATION			AV. WEIGHT AT 2 WEEKS			GLYCINE TOXICITY SYMPTOMS
Folacin	Amino acid		Trial C-8	Trial C-12	Trial C-11	
mg/kg			*gm*	*gm*	*gm*	
		None	96	81	75	..
	5.0%	Glycine	69	57	55	Yes
	5.0%	DL-Serine	92			No
	5.0%	DL-Alanine		59		No [1]
	5.0%	L-Tyrosine			56	No
10.0		None	127	93	98	..
10.0	5.0%	Glycine	120	101	91	No
10.0	5.0%	DL-Serine	126			No
10.0	5.0%	DL-Alanine		70		No [1]
10.0	5.0%	L-Tyrosine			64	No

[1] Slow painful gait.

in the presence of added folacin. Larger quantities of sodium formate were found to be toxic and did not respond to folacin supplementation. Furthermore, dietary formate failed to influence the toxic effect of glycine.

Figure 1 suggests a relationship between dietary riboflavin and glycine toxicity. While growth on the glycine-containing

ration was poor at all riboflavin levels, symptoms of glycine toxicity were less severe at the lower levels of riboflavin.

The effects of single doses of glycine, glyoxylic acid and oxalic acid on chicks are shown in table 5. Glyoxylic acid and

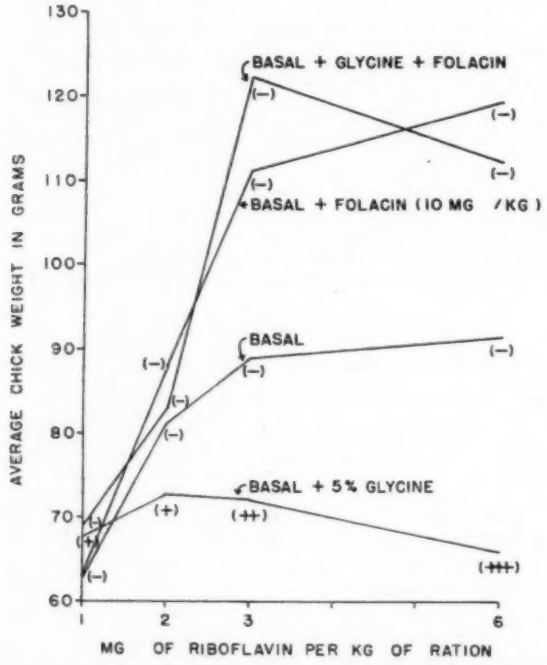

Fig. 1 Effect of dietary riboflavin level on glycine toxicity (exp. C-16). Minus sign (—) indicates absence of toxicity, plus sign (+) indicates presence of toxicity symptoms and severity of symptoms is indicated by increased number of plus signs (++) or (+++).

oxalic acid proved to be toxic in smaller amounts than glycine, but with symptoms different from those produced by glycine. Dietary folacin failed to protect the chicks against glyoxylic or oxalic acid, but as usual helped the chicks withstand an otherwise lethal dose of glycine.

The feeding of ascorbic acid, niacin, riboflavin, pyridoxine, vitamin B_{12}, and alpha tocopherol in addition to the quantities of these vitamins contained in the basal ration had no effect on glycine toxicity where folacin-deficient rations were employed.

TABLE 4

Effect of sodium formate on chick growth and on glycine toxicity

GROUP NO.	SUPPLEMENT TO BASAL RATION			AV. WEIGHT AT 2 WEEKS			GLYCINE TOXICITY SYMPTOMS
	Folacin	Glycine	Na formate	Trial C-14	Trial C-15	Trial C-16	
	mg/kg	%	%	*gm*	*gm*	*gm*	
1				83 [1]	95 [1]	79 [1]	..
2		5.0		59	71	..	Yes
3			2.0	93	104	94	..
4		5.0	2.0	..	67	..	Yes
5			4.0	80	..	..	..
6	10.0			120	125	127	..
7	10.0	5.0		105	112	..	..
8	10.0		2.0	106	115	119	..
9	10.0	5.0	2.0	..	107	..	..
10	10.0		4.0	85	..	..	..

[1] Analysis of variance, combining three trials, indicates differences statistically significant at 1% level between group 1 and groups 3, 6 and 8.

TABLE 5

Effect of dietary folacin on the toxicity of glycine, glyoxylic acid and oxalic acid given in a single oral dose

(Exp. C-17)

DOSE AS % OF BODY WEIGHT	BASAL		BASAL + 10 MG FOLACIN/KG	
	% Survivors (48 hours after treatment)	Av. gain or loss in weight of survivors	% Survivors (48 hours after treatment)	Av. gain or loss in weight of survivors
		gm		*gm*
None	100	+ 14	100	+ 21
Water	100	+ 14	100	+ 21
0.5% Glycine	100	+ 3	100	+ 12
1.0% Glycine	0	..	80	+ 5
0.1% Glyoxylic acid	60	— 5	60	— 5
0.2% Glyoxylic acid	0	..	0	..
0.1% Oxalic acid	0	..	0	..
0.2% Oxalic acid	0	..	0	..

DISCUSSION

The present experiments do not settle the question as to why glycine is toxic, nor do they reveal exactly how folacin counteracts this toxicity. They do, however, restrict the possibilities somewhat. The toxicity does not appear to be due to high levels of blood glycine *per se,* since the levels of blood glycine were as high in normal chicks fed glycine and folacin as in abnormal birds fed glycine supplements alone. Nor was the effect one of inducing a folacin deficiency, since tissue folacin was at least as high in the glycine-fed birds as in the controls. The specificity of glycine toxicity is apparent from the fact that the symptoms were not duplicated by alanine or tyrosine nor by the metabolites serine, formate, oxalic acid or glyoxylic acid. Most of these latter substances depressed growth, but depression was not corrected by folacin.

The non-toxicity of serine is particularly suggestive. Serine contributed as much excess metabolizable nitrogen as glycine and hence the adverse effect of the latter can hardly be ascribed to an overloading of the mechanism for nitrogen elimination (uric acid production). Furthermore, the ready conversion of glycine to serine (Elwyn and Sprinson, '50), a reaction that requires folacin, raises the possibility that this might be a major means by which excess glycine is metabolized. Totter, Kelley, Day and Edwards ('50) incubated radioactive glycine with liver homogenates from folacin-deficient chicks and recovered 42% of the activity in isolated glycine and 23% in serine. When normal chick liver homogenates were employed, only 15% of the activity was found in glycine, but 36% was found in the isolated serine. The high levels of folacin required to counteract glycine toxicity, and the present finding that folacin levels in liver usually increase with glycine feeding all suggest that the excessive quantities of glycine might be removed by conversion to serine. However, in the present experiments the extent of this type of removal, as pointed out previously, was not sufficient to decrease the levels of blood glycine, and the symptoms of toxicity would therefore seem best explained as the result of alternate meta-

bolic products that accumulate when the conversion of glycine to serine is impaired. A glycine oxidase which requires flavin-adenine dinucleotide has been found in mammalian tissues (Ratner, Nocito and Green, '44), the immediate product being glyoxylic acid, which can go to oxalic acid by an aldehyde oxidase. If these systems were of significance in the chick, they might help explain why symptoms of glycine toxicity were more severe when dietary riboflavin was increased, even though the specific symptoms were not duplicated when toxic levels of glyoxylic and oxalic acids were given orally. It is of interest that variations in the other vitamins tested did not modify the toxicity of glycine.

SUMMARY

1. Although symptoms of glycine toxicity in chicks were prevented by folacin, dietary glycine failed to depress the folacin content of the tissues, nor did folacin depress the level of glycine in the blood. Liver glycogen was depressed by glycine and elevated by folacin; blood glucose, uric acid, calcium and *p*-hydroxphenyl compounds were unaffected by either.

2. Unsuccessful attempts to simulate glycine toxicity with possible metabolites included the administration of oxalic acid and glyoxylic acid, while the simultaneous feeding of glycine and formate failed to alter the course of glycine toxicity. The feeding of methionine and arginine minimized the symptoms but failed to restore growth.

3. Serine was inocuous at levels equivalent to those that produced glycine toxicity. Tyrosine and alanine depressed growth without correction by folacin. Preliminary evidence suggested that high levels of riboflavin might aggravate the the symptoms of glycine toxicity.

ACKNOWLEDGMENTS

We are indebted to the Dow Chemical Company, Midland, Michigan, for the glycine and to the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, for folacin used in these studies.

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MUSCULAR DEGENERATION IN CHICKENS FED DIETS LOW IN VITAMIN E AND SULFUR [1,2]

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(Received for publication February 13, 1956)

During a study on sulfate metabolism (Machlin, '55), white striations were observed in the muscles of the breast and the legs of young chickens fed diets low in sulfur. The lesions resembled those reported by Dam et al., ('52). In the studies presented here the protective effect of cystine and vitamin E reported by these Danish workers was confirmed. In addition the effect of methionine and the antioxidant diphenyl-*p*-phenylenediamine (DPPD) in the prevention of the muscular degeneration and the negative or slight influence of variety of other compounds are reported.

EXPERIMENTAL

In each experiment, day-old, New Hampshire X Silver Cornish crossbred female chicks were fed the basal diet for one week. They were then weighed, divided into groups of approximately equal weight distribution, and fed the experimental diets for three weeks. The birds were reared under electrically heated broders in wire-floor cages in an air-conditioned room. At the end of the experiment the birds were killed and the muscles examined and scored for severity of muscular degeneration. Tissues for histological examination

[1] A preliminary report appears in Poultry Sci., *34:* 1209 (1955).

[2] This project was supported in part by the United States Atomic Energy Commission.

[3] Present address: Monsanto Chemical Company, St. Louis 4, Missouri.

were dissected immediately after death and placed in 8% formalin.

The basal diet which contains 15% casein and 10% gelatin as the protein sources, is essentially the same as that used by Gordon and Sizer ('55), with 2.62 gm $MgCO_3$, 0.37 gm $MnCl_2$ and 0.045 gm copper acetate replacing the corresponding sulfate salts.

This diet contains about 0.54% of methionine, 0.06% of cystine and no appreciable sulfate S. This is less than the recommended level of 0.8% of the sulfur-containing amino acids considered necessary for optimum growth of the young chicken (Almquist, '52). All diets were stored at 2° C. and mixed freshly for each experiment.

RESULTS

Experiments 1, 2 and 3. The original purpose of experiments 1 and 2 was to determine whether the addition of sulfate S to a low-sulfur diet would stimulate the early growth of the chick. These data are reported elsewhere (Machlin, '55). In these experiments the basal diet contained 4% of cottonseed oil and 10 mg/kg of alpha tocopheryl acetate.

At 4 weeks of age most of the birds fed the basal diet had white parallel striations in the breast muscle in the direction of the muscle fibers. Occasionally these lesions were seen in the leg muscles. Other skeletal muscles were affected only rarely. When examined histologically, it was observed that there were wide linear areas of degeneration involving several adjacent muscle fasiculi which accounted for the white or light colored bands noted grossly. The degeneration was a waxy or hyaline type characterized by fragmentation, hyalinization, loss of striation, multiplication of sarcolemnal cells, infiltration by heterophil cells and clumping of fibers into eosinophilic masses. There was some calcification in specimens with a more severe reaction. Both degeneration and regeneration were apparent in single tissues. For instance, in a part of one specimen there was no cellular reaction in some areas but very diffuse atrophy and disappearance of the muscle fibers and cells. In other

parts of this specimen there was active regeneration with proliferating muscle nuclei.

No significant changes were observed in specimens of gizzard or heart muscle or of liver, kidney, pancreas or spleen tissues from chickens with severe muscular degeneration. No evidence of encephalomalacia, edema or exudative diathesis was seen. Moreover, the birds showed no signs of weakness of the leg or breast muscles. Mortality was low in all experiments.

TABLE 1

Incidence of muscular degeneration in 4-week-old chicks fed a diet containing cottonseed oil

SUPPLEMENT	EXPERIMENT		
	1[1]	2[1]	3[2]
None	23/25[3]	12/17	9/10
Na_2SO_4, 0.5%	10/11	6/17	6/10
DL-Methionine, 0.3%	1/15	0/16	1/10
L-Cystine, 0.3%	.....	.....	0/10
dl, α-Tocopheryl acetate, 40 mg/kg	.....	.....	0/10

[1] Basal diet contains 4% cottonseed oil (Wesson oil) and 10 mg/kg of *dl* alpha-tocopheryl acetate.

[2] Basal diet contains 4% cottonseed oil, but no supplemental vitamin E.

[3] Numerator is the number of chicks showing grossly visible muscular degeneration (white striations); the denominator is the number of chicks examined in each group, which in almost all cases also represents the number of survivors.

In the first two experiments there was a high incidence of muscular degeneration in chickens fed the basal diet which was almost completely prevented by the addition of 0.3% of DL-methionine (table 1). Since this diet contains a substantial amount[4] of vitamin E (32 I.U./kg), it was first thought that degeneration was due to a simple methionine (or cystine) deficiency. However, the muscular degeneration did appear similar histologically to that reported for vitamin E-deficient animals (Pappenheimer, '43) and therefore the effect of a high level of vitamin E was tested in the third experiment. In this and several subsequent experiments, it was clearly de-

[4] Based on alpha-tocopheryl values in Harris et al. ('50).

monstrated that muscular degeneration could be completely prevented by an adequate dietary supplement of vitamin E. It would appear possible that the vitamin E found in cottonseed oil is not completely available to the chick or that the dietary requirement of vitamin E for the prevention of muscular degeneration is very high. The addition of 0.3% of L-cystine also completely prevented the lesion. When 0.5% of sodium sulfate was added there was a slight reduction in the incidence of muscular degeneration. All of these observations were confirmed in other experiments.

Experiments with lard as the fat source. In order to reduce the vitamin E level of the diet, lard was substituted for cottonseed oil. Dam et al., ('52) had found that several antioxidants including methylene blue were almost ineffective in the prevention of muscular degeneration. In experiments 4 and 5 DPPD was tested since this antioxidant is reported (Singsen et al., '55) to be effective in the prevention of encephalomalacia. When 0.05% DPPD was added, the incidence of muscular degeneration was not affected appreciably (table 2). However, at a level of 0.1% there was almost complete protection and subsequent experiments showed that higher levels would give complete protection. The addition of 40 mg/kg of the antioxidant Tenox R,[5] had no effect.

Removal of fat from the diet or the addition of tryptophan had no effect on the incidence or severity of muscular degeneration. The data in table 2 also demonstrate that the protective action of methionine is not due to any possible beneficial effects of increased food intake since birds fed a fat-free diet, or a diet containing Tenox-R consumed as much feed as those fed methionine and still had severe muscular degeneration.

Inositol had not been included in the basal diet since this compound is unnecessary for optimum growth of the chick (Machlin, '49). However, inositol was reported to counteract vitamin E deficiency symptoms in chicks (Dam, '44), and

[5] Tenox R, Eastman Chemical Products, Kingsport, Tenn., contains 20% butylated hydroxy anisole, 20% citric acid and 60% propylene glycol.

therefore the effect of this compound on muscular degeneration was tested. It was found that the addition of inositol to the diet had no effect on muscular degeneration (table 2). In this same experiment it was found that the use of a more purified type of casein was also ineffective.

TABLE 2

Incidence and severity of muscular degeneration in chicks fed a diet containing lard [1]

SUPPLEMENTS	INCIDENCE	SEVERITY [2]	FEED CONSUMPTION
			gm/chick
None	31/37	2.4	
dl, α-Tocopheryl acetate, 0.01%	0/25	..	
DL-Methionine, 0.5%	0/11	..	314
Tenox R, 40 mg/kg [3]	12/12	3.6	308
Glucose replaces lard	10/12	3.2	309
DL-Tryptophan, 0.1%	12/12	3.2	
Na_2SO_4, 0.5%	4/10	1.5	
DPPD,[4] 0.05%	9/12	2.0	
DPPD, 0.10%	1/11	3.0	
DPPD, 0.25%	0/13	..	
Inositol, 0.5%	11/13	2.1	
"Vitamin-free" casein	10/12	2.1	

[1] Basal contains 4% freshly rendered lard. The table is a summary of 4 experiments.

[2] Muscles of the breast for each bird scored as follows; 1 to 3 white striations visible 1; over 3 striations visible 2; entire breast area containing striations 3; most of breast area covered with striations most of which are more than 2 mm in width 4. The score for each experimental group is the average of those birds having any evidence of degeneration.

[3] Eastman Chemical Products, Kingsport, Tenn., contains 20% butylated hydroxy anisole, 20% citric acid, 60% propylene glycol.

[4] Diphenyl-*p*-phenylenediamine.

Experiments with "stripped" lard. In a final attempt to reduce the vitamin E level of the diet to a minimum and still have a source of essential fatty acids, a lard "stripped" of almost all vitamin E activity was used as the fat source in the remainder of the experiments.

It has been reported by Schwarz ('51) that the liver necrosis produced in rats fed diets deficient in vitamin E and sulfur

TABLE 3

Growth and incidence of muscular degeneration of chicks fed diets containing "stripped" lard[1]

SUPPLEMENTS	WEIGHT	INCIDENCE
	gm at 4 weeks	
None	250	63/94
E[2]	245	0/21
Inositol, 0.5%	258	19/24
Thioctic acid,[3] 1 mg/kg	256	12/12
Thioctic acid, 2 mg/kg	271	3/12
Thioctic acid, 4 mg/kg	278	12/12
Thioctic acid, 8 mg/kg	233	11/13
E + 0.5% M[2]	349	0/12
E + 0.5% M + 0.5% inositol	357	0/12
E + 0.5% M + thioctic acid, 1 mg/kg	364	0/12
E + 0.5% M + thioctic acid, 4 mg/kg	355	0/12
Taurine, 0.5%	247	10/13
Taurine, 1.0%	241	11/13
Brewers' yeast,[4] 10%	259	15/25
Brewers' yeast, 20%	198	8/13
Brewers' yeast, 30%	144	5/13
DL-Methionine, 0.1%	294	10/12
DL-Methionine, 0.2%	306	5/13
DL-Methionine, 0.3%	322	1/13
L-Cystine,[5] 0.08%	290	7/13
L-Cystine, 0.16%	302	4/13
L-Cystine, 0.24%	315	0/13
DPPD,[6] 0.05%	237	6/11
DPPD, 0.10%	243	2/13
DPPD, 0.15%	238	2/8
DPPD, 0.25%	238	0/13
Chlorotetracycline, 0.01%	290	8/10

[1] Basal contains 2% "stripped" lard. Distillation Products Industries, Rochester, N. Y. 500 mg/kg of Tenox R was added to the lard immediately after opening each can. The table is a summary of 5 experiments.

[2] E = 0.01% *dl* alpha tocopheryl acetate, M = DL-methionine.

[3] Furnished by Dr. E. L. R. Stokstad, Lederle Laboratories, Pearl River, N. Y.

[4] Brewers' yeast composite furnished by Elsie Singruen, Brewers' Yeast Council, Inc., Chicago, Ill., The yeast replaces equal parts of casein and glucose.

[5] Equivalent to comparable methionine on a sulfur basis.

[6] Diphenyl-*p*-phenylenediamine.

can be prevented by addition of a factor (Factor 3) present in brewers' yeast. Moreover Scott et al., ('55) found that the addition of brewers' yeast to a diet low in vitamin E prevented exudative diathesis. The data (table 3) demonstrated that brewers' yeast at levels as high as 30% did not prevent muscular degeneration. A sample of yeast was fed which was known to be effective in the prevention of exudative diathesis under the conditions employed by Scott et al. ('55).[6]

The data in table 3 also show that inositol and thioctic acid have no effect on muscular degeneration. Moreover they have no effect on growth when added to the basal diet or to diets containing vitamin E and methionine. Taurine, a sulfur-containing compound, was also without effect.

Chlorotetracycline, which was reported to aid in the prevention of liver necrosis in rats (Gyorgy et al., '51) had no effect on the development of muscular degeneration in the chicken.

Methionine and cystine appear to be approximately equal in protective activity. This is in contrast to the report (Schwarz, '54) that methionine is only 35% as active as cystine for the prevention of liver necrosis.

Injection of DPPD. In order to determine whether the site of the action of DPPD was in the intestinal tract or in the body of the chicken, DPPD was injected. A suspension of DPPD in 0.5% Tween-80 was injected into the leg muscle three times weekly so as to supply the equivalent of chicks receiving 0.01% or 0.05% DPPD in their ration. The antioxidant completely prevented muscular degeneration at the 0.05% level and only two out of 8 chicks were affected at the 0.01% level. It would therefore appear that the primary site of its function is in the tissues of the body.

DISCUSSION

Although muscular degeneration is not a common manifestation of a vitamin E deficiency in chickens (Pappenheimer, '43) it has been observed in ducks (Pappenheimer

[6] Scott, M. L., personal communication.

and Goettsch, '34; Gerriets, '54) and pheasants (Jungherr et al., '49). It appears however from the work of Dan et al. ('52), and that reported here, that muscular degeneration does occur in chickens if the diet is also deficient in sulfur. Since it is quite possible that the diets used in the studies with ducks were deficient in cystine and methionine it is possible that the difference in response to vitamin E-deficiency between these species is not as distinct as once thought.

Our work would indicate that the total requirement for methionine and cystine for the prevention of muscular degeneration is approximately the same as that required for optimum growth. The reduction in the incidence of muscular degeneration resulting from the addition of sodium sulfate is probably due to the action of sulfate in sparing the requirement of the chicken for the sulfur-containing amino acids (Gordon and Sizer, '55; Machlin, '55). The protective effect of cystine and methionine against muscular degeneration is in contrast to the lack of their effect on exudative diathesis (Dam et al., '51; Scott et al., '55), or of cystine or methionine on encephalomalacia (Singsen et al., '55).

The addition of brewers' yeast to the diet did not increase growth or affect muscular degeneration, whereas this supplement did increase growth and prevented exudative diathesis under the conditions employed by Scott et al. ('55). The reason for these differences is not clear, nor is there any obvious explanation for the fact that only muscular degeneration occurred in chicks fed diets low in vitamin E and sulfur, whereas exudative diathesis was observed by the Cornell workers in chicks fed diets deficient in these same nutrients. It is possible that the Torula yeast used by these workers is deficient in ingredients such as Factor 3 or available amino acids which are present in a casein-gelatin type of diet, or that this yeast contains dietary factors (e.g. fatty acids, purines, pyrimidines) which strongly influence the manifestation of a vitamin E deficiency.

The studies presented here show that the requirement for DPPD for the prevention of muscular degeneration is be-

tween 0.15% and 0.25% of the diet. This is much higher than the level of 0.025% found to be adequate for complete protection of encephalomalacia (Singsen et al., '55). DPPD was also effective when injected. Although DPPD might function by the protection of small amounts of vitamin E present in the basal diet or in the tissues of the bird, the studies of Singsen et al. ('55) on the effect of DPPD on blood tocopheryl suggest that a dietary level of much less than 0.15% would suffice for such a function. Until more is known about the mechanism of action of antioxidants, it will be difficult to explain how DPPD can prevent muscular degeneration or why there is a higher level of this antioxidant needed for the prevention of muscular degeneration compared to the requirement for protection against encephalomalacia.

SUMMARY

Chickens fed diets low in vitamin E and sulfur to 4 weeks of age developed a muscular degeneration manifested grossly as white striations of the breast and leg muscles, and microscopically as a hyaline type degeneration. No significant changes were noted in the tissues of the gizzard, heart, liver, pancreas, kidney or spleen.

The addition of alpha tocopheryl-acetate, methionine, cystine, or a high level of diphenyl-p-phenylenediamine (DPPD) (0.25%) to the diets completely prevented muscular degeneration. Addition of 0.5% of sodium sulfate slightly reduced the incidence of muscular degeneration. However supplements of brewers' yeast, tryptophan, chlorotetracycline, inositol, taurine or thioctic acid had no effect on this pathological alteration.

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A STUDY OF THE EFFECT OF CHLORTETRACYCLINE (AUREOMYCIN) UPON CALCIUM RETENTION BY THE GROWING MALE ALBINO RAT [1]

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(Received for publication March 20, 1956)

Numerous theories have been advanced to explain the stimulation of animal growth given by antibiotics. One of the more prominent theories suggests that the antibiotic induces increased absorption of nutrients from the gastrointestinal tract. If this were the main mode of action, it would be expected that mineral absorption would be increased and a greater amount of ingested calcium (Ca) would be available to the animal for the laying down of bone tissue. Migicovsky et al. ('51) reported that dietary penicillin significantly increased the Ca absorption index of chicks which had previously been fed a low Ca diet. Ross and Yacowitz ('54) found that dietary procaine penicillin G significantly increased chick bone (tibia) ash. Rusoff et al. ('54) reported larger skeletal growth and increased bone size in young calves to which aureomycin was administered parenterally or orally. Murray and Campbell ('55) found the response of rachitic rats to doses of vitamin D, as measured by the line test (U.S.P. XIV, '50), was increased by the addition of aureomycin to the diet.

The present study was undertaken to test whether aureomycin could, during a 5-week growth period, increase the re-

[1] Authorized on 3/7/56 for publication as paper no. 2050 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

tention of carcass calcium in weanling rats given experimental diets containing two levels of Ca (0.55% and approximately one-half of this amount) in the presence of 0.44% of inorganic phosphorus and adequate vitamin D under the condition of equal food intakes within replicates of animals.

EXPERIMENTAL

Forty weanling male albino rats weighing 42 to 64 gm and housed in individual screen-bottom cages were assigned on the basis of body weight and litter to 10 replicates of 4 animals each. All rats within a replicate received daily quantities of experimental diets equal to the consumption of the smallest eater.[2] Rat 1 of each replicate *(basal)* received a ration containing, in percentage, vitamin-test casein,[3] 18; DL-methionine, 0.2; corn oil,[4] 7; cornstarch, 40; sucrose, 10; cerelose, 18.68; vitamin mix,[5] 0.5; choline chloride, 0.1; cellulose, 2; modified Salts IV (Ca-free),[6] 2.85; and $CaCO_3$, 0.67. Rat 2 of each replicate *(basal + aureomycin)* received a diet identical to that given rat 1 to which aureomycin had been thoroughly admixed at the rate of 100 mg/kg of diet. Rat 3 of each replicate *(basal + Ca)* received a diet the same as that given rat 1 except that 4% of Salts IV [6] replaced the modified Salts IV and $CaCO_3$. Rat 4 of each replicate *(basal + aureomycin + Ca)* received a diet identical to that given rat 2 to which aureomycin had been thoroughly admixed at the rate of 100 mg/kg of diet. The additional minerals of the latter two diets were added at the expense of cerelose. By analysis

[2] It was long ago pointed out by Fairbanks and Mitchell ('36) that "the control of food intake in calcium metabolism studies is essential to the most significant results."

[3] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[4] Oleum Percomorphum (Mead Johnson & Co., Evansville, Indiana) and α-tocopheryl acetate added to the corn oil so that the final diet contained 2,000 I.U. vitamin A 283 I.U. vitamin D, and 10 mg vitamin E per 100 gm.

[5] The vitamin mix had the following composition: thiamine hydrochloride 500 mg, riboflavin 600 mg, pyridoxine hydrochloride 300 mg, Ca pantothenate 3 gm, nicotinic acid 6 gm, folic acid 100 mg, vitamin K 100 mg, vitamin B_{12} 5 mg, biotin 10 mg, and cerelose to make 500 gm total.

[6] Lichstein et al. ('46).

the above diets contained in the order given 0.291, 0.286, 0.549, and 0.551% Ca. Additional vitamin B_1 (50 mg/rat/day as a cerelose premix) was added to each daily portion of diet. This was done in order to replace possible losses of the vitamin by components of the salt mixture, as shown by Waibel et al. ('54). Distilled water was given to all rats ad libitum.

After 35 days on the experiment the animals were killed with chloroform, their body lengths from tip of nose to base of tail were measured, the gastrointestinal tracts were emptied, the carcasses weighed, cut into small pieces and dried in vacuum desiccators over concentrated H_2SO_4 for approximately 10 days. The fat was removed from the dry carcasses by 48 hours extraction with diethyl ether in a Soxhlet apparatus and the dry residue was finely ground in a burr mill. Thus carcass fat and dry matter were measured directly, but carcass water was determined indirectly. Calcium was determined on a 5 gm sample of the finely ground dry residue by the A.O.A.C. ('50) method. Eight litter-mate rats were killed at the beginning of the experiment and were dried, extracted, ground and analyzed in an identical manner.

RESULTS AND DISCUSSION

Table 1 summarizes the experimental results obtained, showing group means and the standard error of the means for the various criteria used to evaluate the experiment. Column 1 of the table contains the items tested: body weight gain ("empty" carcass weight — initial body weight); carcass dry matter gain, carcass water gain, and carcass ether extract gain (respectively, the content of these substances in the "empty" carcass — the initial amounts present); carcass Ca gain (Ca content of the "empty" carcass — initial Ca); and percentage Ca retention [(carcass Ca gain/Ca intake) × 100]. In order to arrive at initial dry matter, water, ether extract, and Ca values, initial body weights were multiplied by the mean percentages (20.4, 66, 3.99, and .85, respectively) found for these substances in the carcasses of the control rats killed at the beginning of the experiment. Columns 2, 3, 4, and

5 show mean values obtained for groups 1, 2, 3, and 4, respectively. Column 6 shows the standard errors of the means. The number of animals in each group was originally 10, but the data given in table 1 cover, in some cases, smaller numbers. In one instance an animal escaped from its cage for approximately 12 hours and was removed from the experiment; another animal died from unknown cause and was discarded;

TABLE 1

Effect of aureomycin on growth and calcium content of the body

ITEM	GROUP 1	GROUP 2	GROUP 3	GROUP 4	STANDARD ERROR OF THE MEANS
	Basal (9)[1]	Basal + aureomycin (10)[1]	Basal + Ca (9)[1]	Basal + aureomycin + Ca (10)[1]	
	mean	*mean*	*mean*	*mean*	
Body length, cm	21.42	21.33 [2]	21.38	21.27	± 0.15
Body weight gain, gm	180.9	180.8	176.8	175.1	± 6.3
Carcass dry matter gain, gm	44.51 [2]	42.81 [2]	43.27	41.89	± 1.44
Carcass water gain, gm	114.78 [2]	111.59 [2]	106.94	102.64	± 3.48
Carcass ether extract gain, gm	31.98 [2]	34.84 [2]	31.61	35.61	± 2.38
Carcass Ca gain, gm	1.092	1.095	1.356	1.323	± 0.045
Ca retention, %	77.3	79.1	51.7	49.7	± 2.0

[1] Number of animals in group.
[2] Number of animals = 8.
[3] Number of animals = 9.

in another instance the body length measurement was inadvertently not recorded; and finally, data for ether extract gain of two animals are not included because there was evidence that an undetermined loss of carcass fat had occurred during the ether extraction.

Analyses of variance were made for the criteria of response given in column 1 of table 1. The interactions of additional dietary Ca with the antibiotic were small and failed in each

case to attain statistical significance. For this reason the variance of the main effects of the treatments and the probability that such variance resulted from chance alone was determined. The results of such statistical treatment appear in table 2. For convenience, the mean values for the control group appear in column 2, and the mean deviations from these values caused by the main effects and interaction, appear in columns 3, 4, and 5, respectively. The main effects

TABLE 2

Main effects and interactions of added calcium and aureomycin

ITEM	CONTROL GROUP	MEAN MAIN EFFECTS		MEAN INTERACTION
	Mean	Calcium	Aureomycin	
Body length, cm	21.42	— 0.05	— 0.10	— 0.02
Body weight gain, gm	180.9	— 4.9 [1]	— 0.9	— 1.6
Carcass dry matter gain, gm	44.51	— 1.08	— 1.54 [1]	0.32
Carcass water gain, gm	114.78	— 8.39 [2]	— 3.74	— 1.11
Carcass ether extract gain, gm	31.98	0.20	3.43 [2]	1.14
Carcass Ca gain, gm	1.092	0.246 [2]	— 0.015	— 0.036
Ca retention, %	77.3	— 27.5 [2]	— 0.1	— 3.8

[1] $P < 0.05$.
[2] $P < 0.01$.
[3] $P \cong 0.051$.

of additional dietary Ca were a significant decrease in body weight gain, a highly significant decrease of carcass water gain, and a highly significant increase of the carcass Ca gain — the latter accompanied by a highly significant decrease in the percentage of Ca retention. The finding of an increased carcass Ca gain accompanied by a decreased percentage of retention when an additional increment of dietary Ca was given is in accord with the previous experience of Fairbanks and Mitchell ('36). The finding of a significant decrease in

body weight gain with the increment of dietary Ca added is largely explained by the highly significant decrease in carcass water gain and is also in accord with the findings of the previously cited authors, since they found that an inverse relation exists between the rate of growth and the Ca content of the carcass.

The main effects of aureomycin addition to the basal diet were slight and non-significant decreases in all of the criteria tested save carcass dry matter gain and carcass ether extract gain. In regard to the former criterion a significant decrease was noted. However, in the case of the latter criterion, the increase of 3.43 gm was indeed close to statistical significance. The tendency toward increased deposition of fat incident to the feeding of antibiotics is in agreement with the work of Black and Bratzler ('52), who used both a crude vitamin B_{12} supplement containing streptomycin and streptomycin without added B_{12}; with the work of Knoebel and Black ('52), who used a similar crude vitamin B_{12} supplement containing streptomycin and terramycin together with additional terramycin and added aureomycin; with the work of Hartsook and Johnson ('53), who used terramycin, but at variance with the work of Forbes ('54), who used streptomycin and chloromycetin. In the references cited, however, it appears that the amount of carcass ether extract gain obtained upon feeding antibiotic (s) stands in inverse relationship to the adequacy of the diets for animal growth.

The finding that increased utilization of Ca over the entire experimental period did not occur in this work with the rat, on supplying aureomycin, as was found in short-term experiments with chicks (Migicovsky et al., '51) and that increased skeletal growth as would be indicated by increased body length was not found in this work as was reported in work with cattle (Rusoff et al., '54) can probably be explained by species differences in response to antibiotics. Species differences in growth responses to antibiotics have been repeatedly noted.

SUMMARY

Ten replicates of 4 weanling, male, albino rats each were fed daily for a 35-day period equal quantities of the following experimental diets: rat 1 received the basal diet (containing approximately 18% casein, 0.29% Ca and 0.44% P), rat 2 received the basal diet + aureomycin (100 mg/kg), rat 3 received the basal diet + additional Ca (0.55% Ca), and rat 4 received the basal diet + additional Ca and aureomycin.

Calcium supplementation of the basal diet resulted in a highly significant increase (22.5%) in carcass Ca gain, a significant decrease (2.7%) in body weight gain, a highly significant decrease (7.3% and 35.6%, respectively) in carcass water gain and in the percentage of Ca retention.

Aureomycin supplementation of the basal diet did not increase body weight gain, carcass water gain, body length, carcass Ca gain, or the percentage of Ca retention. Aureomycin, did, however, increase (10.7%) the carcass ether extract gain to an extent that closely approached statistical significance at the 5% level, and significantly decreased (3.5%) carcass dry matter gain.

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THE PEPSIN-DIGEST-RESIDUE (PDR) AMINO ACID INDEX OF NET PROTEIN UTILIZATION [1]

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(Received for publication March 26, 1956)

Although the nutritional quality of a protein must in the final analysis be established biologically there are many advantages to be derived from an *in vitro* method which accurately predicts the biological value of a protein from its chemical composition. It has been recognized since the investigations of Osborne and Mendel that the nutritional value of a protein is primarily dependent upon its constituent amino acids. Mendel ('23) proposed that the efficiency of the individual protein in this respect must depend on the minimum quantity of any indispensable amino acid that it will yield. On the basis of this concept Mitchell and Block ('46) evolved a workable system for the quantitative evaluation of a protein from its amino acid composition. Using whole egg protein as the standard, the nutritive value of a protein was expressed as a "chemical score," equal to the greatest percentage deficit in an essential amino acid in the test protein. Oser ('51) devised a similar method in which all of the essential amino acid "egg ratios" were integrated by calculation of their geometric mean. With reference to man and the rat both methods of scoring yielded values which correlate well with the biological values [2] of a variety of

[1] A preliminary report of this work was presented before the American Society of Biological Chemists at San Francisco, California, April 11–15, 1955 (Sheffner, Eckfeldt and Spector, '55).

[2] The term biological value is used in this paper in accordance with the formula introduced by Mitchell ('24) after Thomas.

proteins. However, for many proteins, the calculated values did not reflect the quantitative differences in biological value.

Mitchell ('52), while reviewing a half-century of progress in nutritional evaluation of proteins questioned whether a method which depended in its entirety upon the total amino acid composition could predict precisely the biological value of proteins, since many other factors affected the utilization of dietary protein. One of these factors is undoubtedly related to the observation that delayed supplementation of a deficient protein with the lacking amino acids is ineffective in correcting the deficiency (Berg and Rose, '29; Geiger, '47; Henry and Kon, '46). Based upon this fact and upon subsequent work of their own Melnick and co-workers ('46) and Riesen et al. ('47a) proposed that in addition to the total amino acid composition, the rate of release of amino acids from protein by pancreatic digestion was also an important factor in the nutritional quality of a protein. This concept was utilized by Horn et al. ('52) to evaluate the nutritional quality of food proteins by measuring microbiologically the individual amino acids made available by pepsin, trypsin, and hog mucosa. This method gave good correlation with the biological value of cottonseed meal which had been subjected to various degrees of processing; however, there was no indication that it could be used to compare proteins from different sources. Halevy and Grossowicz ('43), using the growth response of *Streptococcus fecalis* to a pancreatic digest of the test protein, and Dunn and Rockland ('47) and Anderson and Williams ('51), using the proteolytic protozoan, *Tetrahymena geleii*, also developed procedures to estimate the biological value of proteins. However, the values obtained with these methods did not correlate well with the biological value of proteins as determined by rat assay.

It was the purpose of the present investigation (1) to determine the relationship between the biological value of food proteins and the patterns of amino acids released by digestive enzymes, and (2) to develop an *in vitro* procedure which could accurately estimate the nutritional value of proteins.

METHODS AND MATERIALS

Pepsin digests were prepared by incubating 1 gm of protein (N × 6.25) with 25 mg of pepsin (U.S.P. 1:10,000) [2] in 30 ml of 0.1 N H_2SO_4 for 24 hours at 37°C. For pepsin plus trypsin digests, the pepsin digests were buffered with 3 gm of KH_2PO_4, adjusted to pH 8.4, and incubated with 25 mg of trypsin (4 × U.S.P. pancreatin),[2] at 37°C. for 24 hours. For subsequent erepsin digestion, samples previously treated with pepsin and trypsin were adjusted to pH 7.8 and incubated with 100 mg of erepsin [2] at 37°C. for 72 hours. The samples were covered with toluene during incubation and stirred occasionally. Enzyme blanks were prepared for each stage of digestion with 10 times the quantity of enzyme used for sample treatment. At the end of the indicated incubation periods the digests were heated in a boiling water bath for 10 minutes, cooled and adjusted to pH 2. One volume of 10% sodium tungstate and one volume of 2/3 N H_2SO_4 were then added for each 8 volumes of digest. The mixtures were allowed to stand for 10 minutes and filtered. The clear filtrates were adjusted to pH 6.8, diluted to the appropriate amino acid concentration, and stored frozen until analyzed. In the case of starchy samples, e.g., white flour, most of the starch was removed by centrifugation before the digest was heated.

Acid hydrolysates for the determination of the total amino acid content were prepared by autoclaving each protein for 16 hours at 120°C. with 20 ml of 2 N HCl per gram dry weight of sample. However, for the measurement of cystine the samples were autoclaved only three hours (Riesen et al., '47b). Alkaline hydrolysates for the assay of tryptophan and tyrosine were prepared using 20 ml of 5 N NaOH per gram dry weight of sample and autoclaving at 120°C. for 5 hours. To eliminate gel formation in the flour samples these were steamed for 30 minutes in 25 ml of 0.1 N HCl prior to hydrolysis with alkali. Individual amino acid analyses were, in general, per-

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

formed by microbiological procedures previously described (Sheffner et al., '48); however, the final volume per tube was 2 ml and the acid produced by the growth of the organisms was measured by means of a quinhydrone electrode and the Cannon titration apparatus. Also, for the determination of cystine the medium and samples were autoclaved separately to minimize destruction of this amino acid. *Leuconostoc mesenteroides* P-60 was used for the assay of cystine, histidine,

TABLE 1

"Essential" amino acids in whole egg and casein

AMINO ACID	WHOLE EGG			CASEIN		
	Total	Pepsin digest [1]	Residue	Total	Pepsin digest [1]	Residue
	mg/gm			*mg/gm*		
Histidine	26.8	0.45	26.4	31.8	0.07	31.7
Lysine	82.8	2.9	79.9	80.8	0.34	80.5
Methionine	31.6	7.2	24.4	30.4	0.16	30.2
Cystine	21.4	0.54	20.9	3.4	0.07	3.3
Phenylalanine	55.4	11.4	44.0	55.4	4.8	50.6
Tyrosine	36.3	5.9	30.4	52.0	6.2	45.8
Leucine	80.7	52.5	28.2	98.4	38.2	60.2
Isoleucine	55.4	38.2	17.2	58.1	5.9	52.2
Valine	66.3	11.7	54.6	72.5	1.1	71.4
Threonine	49.0	25.9	23.1	41.2	12.5	28.7
Tryptophan	13.5	6.8	6.7	11.6	4.0	7.6
	519.2	163.5	355.8	...	73.3	462.2

[1] Corrected for enzyme blank.

lysine, methionine, phenylalanine, tyrosine and valine, and *Streptococcus fecalis* for isoleucine, leucine, threonine and tryptophan. Total nitrogen was measured by the macro-Kjeldahl procedure using mercuric oxide as the digestion catalyst. Alpha amino nitrogen was determined by the copper titration method of Pope and Stevens ('39).

CALCULATION OF THE PDR INDEX

The 8 amino acids essential to human nutrition plus cystine and tyrosine are used when the test protein is evaluated for

man. When the protein is evaluated for the growing rat, histidine is also taken into account. To illustrate the method, calculation will be made of the PDR index of casein for use with the growing rat.

The concentrations of these 11 amino acids in whole egg and in casein are determined in the completely hydrolyzed proteins and in the pepsin digests. The concentration of each amino acid (mg/gm of protein) microbiologically available in the pepsin digest is subtracted from the concentration of the respective amino acid in the total hydrolysate to give the "residue" fraction (table 1).

Each amino acid is then calculated as the percentage of the sum of the 11 amino acids for the protein in the pepsin digest and residue fractions, respectively. For example, the "free" histidine content of the pepsin digest of whole egg is 0.45 mg. The sum of the 11 amino acids in the pepsin digest is 163.5 mg. Histidine thus constitutes 0.45/163.5 or 0.28% of the sum of these 11 amino acids in the pepsin digest (table 2).

The ratio of the percentage of each amino acid in the pepsin digest of casein to the percentage of that amino acid in the pepsin digest of whole egg gives the "egg ratio." The geometric mean of the adjusted egg ratios is then computed logarithmically by averaging the logarithms of the egg ratios, and obtaining the antilogarithm. A similar calculation is made for the residue fraction. Egg ratios less than one are considered to be one in order to avoid negative logarithms. In computing the egg ratios, percentage concentrations of amino acids in excess of those present in the standard protein are disregarded. Methionine and cystine are considered as a unit, as are phenylalanine plus tyrosine. If the essential precursor amino acid of the pair, e.g., methionine, is present in excess of that in egg, the excess can be used to make up the deficiency of the non-essential amino acid, but the reverse is not done. For example, the pepsin digest of casein is credited with only 3.61% of tyrosine, i.e., the same as in whole egg, rather than 8.46%. The total of phenylalanine (6.55%)

plus tyrosine (3.61%) is thus 10.16% and the egg ratio is 10.16/10.58 or 96.0.

The geometric means of the fractions are each multiplied by a factor to correct for the degree of proteolysis of the test protein relative to that for the standard egg protein. The

TABLE 2

Example for calculation of the PDR index for casein

AMINO ACID	PEPSIN DIGEST				RESIDUE			
	Whole egg	Cas.	Egg ratio	Log egg ratio	Whole egg	Cas.	Egg ratio	Log egg ratio
	%	%			%	%		
Histidine	0.28	0.10	35.7	1.5527	7.42	6.86	92.5	1.9661
Lysine	1.77	0.46	26.0	1.4150	22.46	17.42	77.6	1.8899
Methionine	4.40	0.22			6.86	6.53		
Cystine	0.33	0.10			5.87	0.71		
Meth. and cys.	4.73	0.32	6.8	0.8325	12.73	7.24	56.9	1.7551
Phenylalanine	6.97	6.55			12.37	10.95		
Tyrosine	3.61	8.46			8.54	9.91		
Phen. and tyr.	10.58	10.16	96.0	1.9823	20.91	19.49	93.2	1.9694
Leucine	32.11	52.11	100.0	2.0000	7.92	13.02	100.0	2.0000
Isoleucine	23.36	8.05	34.5	1.5378	4.83	11.29	100.0	2.0000
Valine	7.16	1.50	20.9	1.3201	15.34	15.45	100.0	2.0000
Threonine	15.84	17.05	100.0	2.0000	6.49	6.21	95.7	1.9809
Tryptophan	4.16	5.46	100.0	2.0000	1.88	1.64	87.2	1.9405
Av. logarithm of egg ratios =				1.6267				1.9446

Geometric mean:
Pepsin digest fraction = antilog 1.6267 = 42.3
Residue fraction = antilog 1.9446 = 88.0

Corrected geometric mean:
Pepsin digest fraction = 42.3 × 73.3/163.5 = 18.96
Residue fraction = 88.0 × 462.2/355.8 = 114.32

PDR index = antilog (0.315 × log 18.96 + 0.685 × log 114.32) = 64.9

PDR index/digestibility = 64.9/0.97 = 66.9

factor for the pepsin digest is obtained by summing the concentrations (mg/gm) of the 11 individual amino acids in the pepsin digest of the test protein and dividing the total by the sum of the concentrations obtained for the standard egg. For example, the sum of the 11 amino acids in the pepsin

digest of casein is 73.3 and the sum for egg is 163.5. The factor is 73.3/163.5. Similarly, for the residue fraction the factor is 462.2 (casein)/355.8 (egg). Multiplying the geometric mean of the two fractions by their respective proteolysis factors yields the corrected geometric means.

To obtain an amino acid index for the whole protein (casein) the corrected geometric means of the pepsin digest fraction and the residue fraction must be weighted in accordance with

TABLE 3

Comparison of the patterns[1] of the microbiologically available amino acids in enzymatic digests and in completely hydrolyzed whole egg protein and casein

AMINO ACID	WHOLE EGG				CASEIN			
	Pepsin	Pepsin + trypsin	Pepsin + trypsin + erepsin	Total	Pepsin	Pepsin + trypsin	Pepsin + trypsin + erepsin	Total
	%	%	%	%	%	%	%	%
Leucine	33.4	24.6	21.2	17.5	57.0	28.4	25.2	20.5
Isoleucine	24.3	13.6	12.1	12.0	8.8	11.2	10.1	12.1
Threonine	16.5	14.4	13.0	10.6	18.6	13.6	12.8	8.6
Valine	7.4	12.9	14.7	14.4	1.6	8.2	11.5	15.1
Phenylalanine	7.3	11.6	11.3	12.0	7.2	9.1	10.8	11.5
Methionine	4.6	5.6	7.0	6.8	0.2	2.9	3.0	6.3
Tryptophan	4.3	3.9	3.5	2.9	6.0	3.1	3.2	2.4
Lysine	1.8	10.4	13.6	17.9	0.5	21.1	20.9	16.8
Histidine	0.3	3.0	3.6	5.8	0.1	2.3	2.5	6.6

[1] Each amino acid value was calculated as the percentage of the sum of the 9 amino acids indicated.

the percentage each represents of the total standard egg protein. The concentrations of the 11 amino acids measured in the pepsin digest of the standard egg protein represented, on the average, 163.5/519.2 or 31.5% of these amino acids in the entire egg protein, and the amino acids in the residue represented 355.8/519.2 or 68.5% of the 11 amino acids in the entire egg protein. The corrected geometric means are weighted and averaged geometrically to obtain the PDR index for the whole protein.

RESULTS

The patterns of microbiologically available essential amino acids present in enzymatic digests and in complete hydrolysates of whole egg and casein are given in table 3. It can be seen that there are large differences in the proportions of amino acids liberated from the two proteins by pepsin digestion. These differences are considerably reduced when digestion is continued by treatment with trypsin, or trypsin and erepsin. For example, isoleucine represented 24.3% of the 9 amino acids measured in the pepsin digest of whole egg, whereas it was only 8.8% in the pepsin digest of casein. This ratio of 3:1 was reduced to almost 1:1 following treatment with trypsin. Similarly, the proportion of methionine in the pepsin digest of whole egg was 23 times as great as in the pepsin digest of casein. After tryptic action the ratio is diminished by only about 2:1. Leucine, which is present in much smaller proportion in the pepsin digest of whole egg than of casein, is present in almost equal proportions in the tryptic digests of both proteins. When the proteins are hydrolyzed to completion the proportions of the 9 amino acids in the two hydrolysates are almost identical.

Melnick et al. ('46) have reported that similar differences in the rate of release of individual amino acids occur after treatment of proteins with pancreatin; however, the results presented here demonstrate that where proteins are first treated with pepsin, as occurs under physiological conditions, the differences between proteins upon subsequent treatment with trypsin are considerably reduced. These data prompted the hypothesis that if appreciable absorption of the products of peptic digestion occurred, some of the differences in biological value between proteins of comparable total amino acid content could be accounted for by the diversity in pattern of amino acids released by pepsin digestion.

If whole egg protein is considered as the standard, the total essential amino acid patterns in proteins can be compared by computing "egg ratios" (Oser, '51). The integrated essential amino acid egg ratios of a variety of proteins are presented

in table 4. These values are calculated according to the method of Oser ('51), as modified by Mitchell ('54) to include tyrosine but omit arginine in computing the revised index (modified essential amino acid index, MEAA). For many proteins, this total amino acid index does not reflect the actual biological value of the protein. For instance, the MEAA index suggests

TABLE 4

Comparison of the PDR index of food proteins with their biological value and net utilization for the growing rat

PROTEIN [1]	BIOLOGICAL VALUE	DIGESTIBILITY	NET UTILIZATION	PDR INDEX	PDR/DIGESTIBILITY	MEAA INDEX	CHEMICAL SCORE
Whole egg	98 [2]	99	97	100	101	100	100
Egg albumin	97 [3]	100	97	95	95	96	84
Defatted egg (com'l)	87 [2]	97	84	83	86	93	75
Lactalbumin	84 [3]	98	82	82	84	92	70
Soy flour	75 [5]	96	72	71	74	82	44
Casein, Labco	68 [2,3,4,5]	97	66	65	67	92	64
Brewers' yeast	66 [3,6]	93	61	61	66	72	36
White flour	52 [5]	100	52	51	51	65	26

[1] The test proteins used in this study were obtained from the following sources: dried whole egg, Blue Star Foods Co.; egg albumin, Emulsol Corp.; defatted egg, Viobin Corp.; lactalbumin (Labco), Borden Co.; soy flour (Nutri Soy), Archer-Daniels-Midland Co.; casein (Labco, vitamin free), Borden Co.; brewers' yeast, (U.S.P. XII), Nutritional Biochemicals Corp.; and white flour (Pillsbury's Best) Pillsbury Mills, Inc.

[2] Mitchell and Beadles ('50).

[3] Mitchell and Block ('46).

[4] Greaves, Morgan and Loveen ('38).

[5] Chick, Boas-Fixsen, Hutchinson and Jackson ('35).

[6] Mitchell ('48).

that casein and egg albumin have similar biological values; however, it is well established that egg albumin has a considerably higher biological value for the growing rat. Lactalbumin has a much lower biological value than egg albumin; but this is not apparent from the MEAA index.

In general, the MEAA index overestimates the biological value. This fact lends support to the hypothesis that much of the potential nutritional value of many proteins is lost

by the release and absorption of disproportionate amounts of essential amino acids at an early stage in digestion. Consequently, a new amino acid index was devised which takes into account the physiological availability of amino acids during digestion. This index combines the pattern of essential amino acids obtained from analysis of the pepsin digest with the amino acid pattern of the remainder of the protein to produce an integrated index — the Pepsin Digest-Residue (PDR) amino acid index.

TABLE 5

Comparison of the PDR index of food proteins with their biological value and net utilization for adult man

PROTEIN	BIOLOGICAL VALUE	DIGESTIBILITY	NET UTILIZATION	PDR INDEX	PDR/DIGESTIBILITY	MEAA INDEX	CHEMICAL SCORE
Whole egg	100 [1,2]	96	96	100	104	100	100
Egg albumin	91 [3]	101	92	95	94	96	84
Soy flour	73 [4]	90	66	69	77	81	44
Casein, Labco	68 [3]	96	65	63	66	90	64
White flour	41 [4]	97	40	49	51	64	26

[1] Murlin et al. ('44).
[2] Murlin et al. ('48).
[3] Hawley et al. ('48).
[4] Bricker et al. ('45).

Comparison of the PDR amino acid index with the net protein utilization and the biological value of food proteins for the growing rat and for man is presented in tables 2 and 3. Values for the chemical score and the modified essential amino acid index of Mitchell (MEAA) are also given for comparison. It will be noted that the chemical score, based upon the limiting amino acid always underestimates the biological value. While the MEAA index gives values in closer agreement with the biological value, there are many cases where the correlation for the MEAA index is also poor. The PDR indices are found to be in close agreement with the net utilization values of the respective proteins for the growing rat and for man. Therefore, the values obtained by dividing the PDR index by digestibility are closely correlated with the biological

values. The regression lines correlating the biological values with the MEAA indices and the PDR/digestibility values are shown in figure 1. The correlation coefficient, r, of the regression line for the PDR index/digestibility is only slightly better

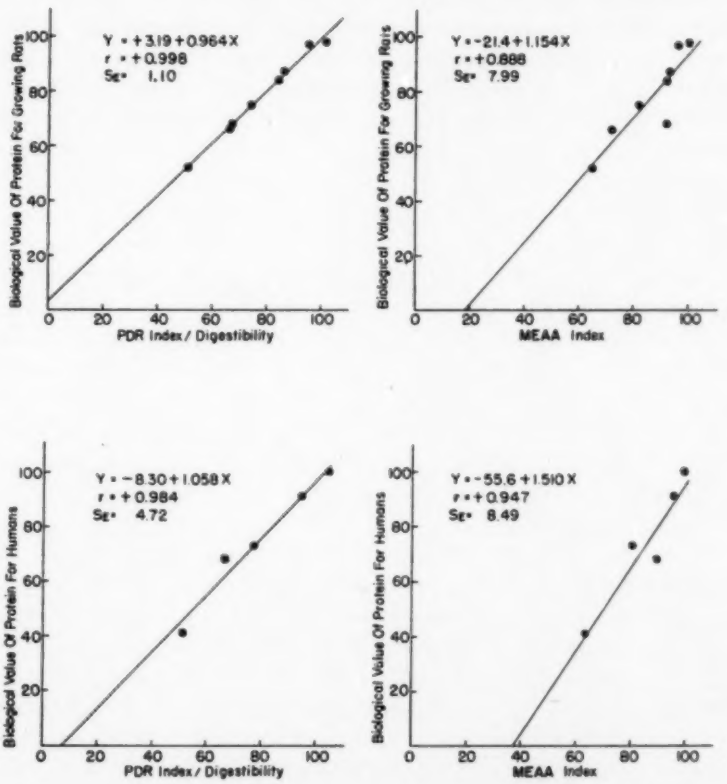

Fig. 1 Relationship between the biological values and (1) the PDR index/digestibility values and (2) the MEAA indices.

than that for the MEAA index since the latter index does, in general, show the proper order of value. However, the regression line for the PDR index/digestibility passes closer to the origin and shows a much smaller standard error of estimate,

S_E, indicating that this index more accurately reflects the quantitative differences in biological value between the various proteins.

DISCUSSION

Following the proposal of Mitchell and Block ('46) whole egg protein was used as the reference protein for the PDR index of net protein utilization. These investigators reported that whole egg protein was almost perfectly utilized in digestion and metabolism for the growing rat. It was demonstrated in the present work that when the well-utilized standard egg protein was treated with digestive enzymes in a manner simulating the physiological sequence, then the pattern of amino acids changed as digestion proceeded. These results suggest that the optimal pattern varies with the stage of digestion or position in the digestive tract. In this respect, many investigators have found that whole protein is better utilized in the diet than protein hydrolysates or amino acid mixtures containing essentially equal quantities of amino acids (Woolley, '46; Womack and Rose, '46; Sheffner, Kirsner and Palmer, '50; Maddy and Swift, '52). Therefore, it appears that the standard of protein quality cannot be a non-varying pattern of amino acids.

The conditions established for obtaining *in vitro* pepsin digests were determined from feeding experiments with rats which indicated that approximately 30% of ingested egg protein nitrogen is absorbed before the chyme has reached the area of the intestine where tryptic activity is significant. Consequently, the quantity of pepsin used and the duration of incubation in the *in vitro* procedure were adjusted to produce approximately 30% release of microbiologically available essential amino acids from egg protein. The quantity of amino nitrogen released, when 1 gm of egg protein is digested with 25 mg of pepsin for 24 hours is almost 90% of that which occurs when either the amount of pepsin or the incubation time is doubled. With pepsin of the proper activity the conditions are such that small variations in time, temperature, and quantity of enzyme will not cause significant variation in

the PDR index. The activity of the pepsin used in these experiments was U.S.P. 1–10,000. Use of 3 × crystallized pepsin [4] resulted in a more rapid release of amino acids but did not change the pattern of amino acids made available.

The excellent correlation between the PDR index and the net protein utilization value of representative proteins suggested that this index should be a valuable *in vitro* method for predicting the nutritional quality of proteins. However, aside from this practical aspect the observed correlations suggest the importance of peptic digestion for optimal utilization of dietary proteins. In this respect, Mellander ('55), Kotschneff ('26, '28), and Sheffner et al. ('50) have suggested that the wall of the intestine is permeable to amino acid complexes. Consequently, it appears reasonable to suggest that an appreciable quantity of the products of pepsin digestion are absorbed from the duodenum before pancreatic enzymes can act on them, and that the pattern of these absorbed amino acids and peptides is an important factor in the efficient utilization of ingested protein.

The extent to which the assay microorganisms used in this study measured peptides or larger amino acid complexes is not known. However, the PDR indices could not be correlated with their respective biological values if the large polypeptides in the protein-free (tungstic acid filtrates) pepsin digests were hydrolyzed before the digests were analyzed. In this connection, Sheffner, Kirsner and Palmer [5] found that when humans were fed a protein-containing meal, the concentrations of microbiologically non-available polypeptide amino acids of plasma, i.e., large polypeptides, decreased after the meal, whereas concomitantly the microbiologically available plasma amino acids (free amino acids and small polypeptides) were significantly increased. These data suggest, therefore, that if polypeptides are absorbed at an early stage of digestion, they probably consist of relatively few amino acid residues.

[4] Pentex Incorporated, Kankakee, Illinois.

[5] Sheffner, Kirsner and Palmer, unpublished data.

It is apparent that the last step in the calculation of the PDR index predicts that any process which severely decreases peptic digestibility will lower the nutritional value of the treated protein in spite of patterns of subsequently released amino acids which are equally good. The significance of this fact is not yet understood. However, work is continuing to determine the nutritional importance of amino acid patterns during the early stages of absorption, and the effect of peptic digestion upon the subsequent digestion and utilization of protein.

SUMMARY

The relationship between the pattern of amino acids released by digestive enzymes and the biological value of food proteins was studied. The pattern of amino acids released *in vitro* by pepsin revealed differences between proteins which were not apparent from their total essential amino acid content nor from the patterns existing when the pepsin digests were further digested with trypsin and erepsin.

An amino acid index is described which takes into account the physiological availability of amino acids during digestion. The new index combines the pattern of essential amino acids released by *in vitro* pepsin digestion with the amino acid pattern of the remainder of the protein to produce an integrated index — the Pepsin-Digest-Residue (PDR) amino acid index. The PDR index was closely correlated with the net protein utilization value of a variety of proteins. Division of the PDR index by the digestibility coefficient of the respective proteins yielded values which accurately predicted the biological values of the proteins studied.

ACKNOWLEDGMENT

The authors wish to thank Mr. John McMullen for providing the proximate analyses and Mr. Richard R. Adachi for some of the amino acid analyses.

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INFLUENCE OF FOOD AND ENERGY RESTRICTION AND SUBSEQUENT RECOVERY ON BODY COMPOSITION AND FOOD UTILIZATION OF RATS

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Most animals at some time during their life cycle are likely to undergo periods in which dietary restrictions lower the growth below the maximum obtainable. The influence of diet on life span has been recently reviewed by Silberberg and Silberberg ('55). Osborne and Mendel ('14) first reported that suppression of the growth of rats did not erase the capacity to grow. Clarke and Smith ('38) noted that caloric restriction for three weeks would not prevent rats from attaining the size of the controls. Earlier, Stewart ('16) had also noted that after short periods of starvation rats do have the ability to attain the control size. Factors such as prolonged periods of stunting (Clarke and Smith, '38; Quimby, '48; Jackson and Stewart, '20; and McCay et al., '35, '39) and growth restrictions during suckling (Schultze, '55; and Jackson and Stewart, '20) have not allowed animals to reach full size after a period of liberal feeding. The literature has been reviewed on cattle and interrupted growth by Winchester and Howe ('55) and they have also shown that steers restricted in their energy intake and then refed would attain the size and approximate composition of the controls. The restricted steers made a weight gain equal to that of the controls on about the same amount of feed even though it took a longer period of time.

The influence of nutrient restriction and subsequent recovery on body composition of simple-stomached animals has not previously been studied nor has much attention been given to the total amount of food required due to the interruption of growth and subsequent recovery. Therefore, these experiments were designed to study these factors and to attempt to define experimental conditions more rigidly. This was done by studying both a total food and energy restriction with purified diets and by controlling food intake on the basis of body weight$^{0.75}$ (Kleiber, '47).

EXPERIMENTAL

The experimental design, ration composition, and food intake in experiment 1 are illustrated in table 1. The rats of lot 1, the control, received the basal ration ad libitum for the entire experimental period. The food intake of the animals in lot 2 was restricted for 28 days, and then they were fed the basal ration ad libitum until they had eaten an amount equal to that consumed by lot 1. Lot 3 was fed in a similar manner to lot 2, except that the rats were restricted in energy intake (sucrose and cottonseed oil) but given an intake of protein, minerals, and vitamins equal to lot 1. The degree of food or energy restriction was 70% of the intake of lot 1, per unit of metabolic body size (body weight$^{0.75}$; Kleiber, '47). Therefore, the actual food intake of lot 2 and the energy received by lot 3 were decreased each week so that each was less than 70% of the amount received by lot 1. These factors, however, remained at 70% on the basis of body weight$^{0.75}$. This was done because of the greater weight increase of the rats in lot 1 and hence a greater maintenance requirement than for lots 2 and 3. The animals of lots 2 and 3 were thus pair-fed with lot 1.

Eighteen male weanling rats (Sprague-Dawley strain) were used in each group. Nine in each group were killed for body composition studies at the end of the restriction period. The remainder were killed at the end of the experiment. Body composition was determined on the body minus the gastro-

intestinal tract and contents. As suggested by Pace and Rathbun ('45) and confirmed by Meyer ('55), body water was determined and the fat-free body calculated from the body water content. The constant of 73.5% water in the fat-free body was used.

The feeding in experiment II was conducted in the same manner as in experiment I except that the food restriction was for 21 days. Weighing conditions were changed slightly. To nullify the influence of the gastrointestinal content on body

TABLE 1

Experimental design and food consumption in experiment I

CONSTITUENTS OF DIET	LOT 1 Controls	LOT 2 70% food consumption of lot 1 [1]	LOT 3 70% energy consumption of lot 1 [1]
	gm	*gm*	*gm*
Average food consumption during restriction period			
Sucrose	173.6	92.2	65.2
Cottonseed oil	12.4	6.6	4.6
Crude casein	49.6	26.4	49.6
Vitamin mix [2,3]	2.5	1.3	2.5
Salts IV [4]	9.9	5.3	9.9
Total	248	131.8	131.8
Average food consumption during recovery period			
Sucrose	170.6	250.7	252.1
Cottonseed oil	12.2	17.9	18.0
Crude casein	48.8	71.5	72.0
Vitamin mix	2.4	3.6	3.6
Salts IV	9.8	14.3	14.4
Total	243.8	357.4	360.1
Grand total	491.8	489.2	491.9

[1] The food consumption of these lots was 70% of the controls per unit of body weight$^{0.73}$.

[2] The vitamin mix contained the following vitamins in milligrams per 100 gm; thiamine 30, riboflavin 30, pyridoxine 20, niacin 200, calcium pantothenate 200, folic acid 3, biotin 1, and vitamin B_{12} 0.2. Sucrose was added to equal 100 gm.

[3] Ample amounts of vitamins A, D, E, and K were supplied weekly by oral administration.

[4] Phillips and Hart, J. Biol. Chem., *109:* 657 (1935).

weight, the rats were kept 12 hours without food before weighing at the start and at the end of the experiment. In addition, the entire body (including gastrointestinal tract and contents) was analyzed for water. Koch ('56) noted no difference in body composition between rats kept 12 hours without food and those analyzed with the intestinal contents removed.

The analysis for variance for paired data (Snedecor, '46) was the statistical treatment for comparison.

RESULTS AND DISCUSSION

Table 2 presents the two experiments. These results were similar. Reduction in growth due to either an energy or total food restriction was about equal. An analysis of the carcass for fat and water and the calculation of fat-free tissue, showed that there were no differences between the two deficiencies. This indicates that the primary deficiency of the total food restriction is one of energy as far as can be measured by gains or body composition.

After realimentation, when the restricted rats were allowed to consume a total amount of food equal to that consumed by the controls, a remarkable ability to recover from this growth interruption was demonstrated. In the first experiment with the 4-week restriction period, both groups of "deficient" rats came within 9 gm of the weight of the ad libitum-fed controls. However, in the second experiment, with a three-week food restriction, there was no statistical difference between the controls and their restricted groups in respect to weight gains. These results are in agreement with those of Clarke and Smith ('38) and Quimby ('48). The work of Winchester and Howe ('55) showed that little extra feed was required by their energy-restricted steers in comparison with the full-fed steers.

The fat and fat-free tissue content of the carcasses or bodies was the same for the rats restricted in energy and those fed ad libitum. This was not true, however, for the rats restricted in total food. These animals, while about the same in weight, had a greater amount of fat and a lesser amount of fat-free tissue. Apparently an advantage is gained for animals during

TABLE 2

Effect of food and energy restriction and of realimentation upon body composition and food utilization in rats

	FOOD RESTRICTION			FOOD RESTRICTION PLUS REALIMENTATION		
	Lot 1 Controls	Lot 2 Food restriction	Lot 3 Energy restriction	Lot 1 Controls	Lot 2 Food restriction	Lot 3 Energy restriction
EXPERIMENT I						
No. of days	28	28	28	42	51	55
Rats per group	18	18	18	9	9	9
Mean gain, gm	114	39 [1]	40 [1]	186.3	177.1 [2]	176.8 [2]
Mean final wt., gm	167	93 [1]	93 [1]	239.1	231	229.7
Carcass data:						
Carcass analyzed	9	9	9			
Mean carcass wt., gm	151.4	86 [1]	83.7 [1]	221.4	213.2	214.7
Fat:						
Wt., gm	16	4.9 [1]	4.9 [1]	25.5	29.4	24.5
Percentage [3]	31	19.1 [1]	19.5 [1]	33.6	38.5 [2]	33.1
Dry fat free body:						
Wt., gm	34.5	20.7 [1]	20.1 [1]	49.9	46.4 [1]	48.5
Percentage [3]	68.4	80.9 [1]	80.5 [1]	66.4	61.5 [2]	66.9
EXPERIMENT II						
No. of days	21	21	21	35	42	41
Rats per group	12	12	12	6	6	6
Mean food consumed, gm	212.3	128.4	128.4	444.8	444.1	442.9
Mean gain, gm	73.2	35.5 [1]	43.3 [1]	149	146.8	140.2
Mean final wt., gm	139.7	104.1 [1]	112.7 [1]	216	215.4	209.6
Composition:						
Rats analyzed	6	6	6			
Fat:						
Wt., gm	11.3	4.3 [1]	4.2 [1]	22.5	24.7	21.8
Percentage [3]	25.4	14.6 [1]	13.1 [1]	30.9	33.6	31.3
Dry fat free body:						
Wt., gm	32.7	25.4 [1]	27.8 [1]	49.5	48.6	47.8
Percentage [3]	74.6	85.4 [1]	86.9 [1]	69.1	66.4	68.7

[1] Highly significant difference from controls.

[2] Significant difference from controls.

[3] Dry basis.

growth interruption if the consumption of protein, vitamins, and minerals is maintained at or above their requirements. This was not apparent at the end of their period of growth restriction but was at the end of the realimentation.

It has been well established that maintenance requirements decrease as undernutrition progresses (Stewart, '16; Thompson and Mendel, '18; Jackson, '37; Forbes et al., '38; and Quimby, '48). This may be due to a lower basal metabolism (Horst et al., '34) or increased digestibility of food (Quimby, '48), or both. Restricted rats in these experiments were able to make gains similar to those of the ad libitum-fed controls when given an equal total food intake even though the total time on experiment was longer. This seems to indicate that the lowered maintenance requirement from restricted feeding was prolonged into the unrestricted feeding period. Winchester and Howe ('55) suggested this for steers. Also it has been reported by Horst et al. ('34) that lowered basal metabolism induced by starvation continued about 7 days after adequate nutrition was initiated.

SUMMARY

Growing rats were restricted in their food or energy intake for three or 4 weeks at 70% of the food intake per unit of body weight$^{0.75}$ of the ad libitum-fed controls. They were subsequently fed a total amount of food equivalent to that consumed by the controls. The rats in the first experiment in which the food and energy intake were restricted for 4 weeks came within 9 gm of the weight gain of the controls. This was accomplished even though the experimental period was longer for the restricted rats. In the second experiment there was statistically no difference between groups in weight gains.

Even though there was little difference in weight gains between rats restricted either by energy or total food intake, differences were apparent in body composition. The proportion of fat to fat-free tissue for the rats restricted in energy intake and subsequently refed was the same as that of the

ad libitum-fed controls. However, a greater proportion of fat was found in the bodies of the rats which were restricted in total food intake and subsequently fed adequately.

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EFFECT OF ADDED LYSINE ON GROWTH OF RATS FED A CEREAL AND MILK DIET

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(Received for publication April 12, 1956)

INTRODUCTION

Studies by Rosenberg and co-workers ('52, '54) have shown that the addition of L-lysine to a bread diet supplemented with fat, salts and vitamins, increases the rate of growth of weanling rats. The bread was made with wheat flour and contained three parts of nonfat milk solids and two parts of yeast per 100 parts of flour. A deficiency of lysine was to be expected in a diet composed mainly of wheat flour. Many workers in nutrition since Osborne and Mendel ('14) have shown that the proteins of wheat and other grains are low in lysine. Since these grains are also used in the manufacture of cereals, which are almost invariably eaten with milk, a study was set up to determine whether the addition of L-lysine to a cereal and milk diet would enhance its nutritive value. E. B. Hart ('52) pointed out the shortcomings of cereal proteins but since they are served with milk, he recommended that the protein value of the combination should be considered since the milk would dissipate any inferiority of the cereal in respect to its protein.

In the present experiments the effect of addition of L-lysine to a diet of cereal [1] and powdered whole milk on the growth of male weanling rats was determined. One ounce of this cereal is usually mixed with 5 oz. of fluid milk in infant feed-

[1] The cereal used in these diets was Pablum Mixed Cereal (Mead Johnson and Co.), consisting of wheat meal (farina), oatmeal, yellow corn meal, wheat germ, tribasic calcium phosphate, powdered alfalfa leaf, dried yeast, sodium chloride and reduced iron.

ing. In the present experiment the basal diet contained 70% of cereal and 30% of powdered milk, equivalent to a mixture of 1 oz. of cereal and 3.5 oz. of fluid milk.

EXPERIMENTAL

Six groups of 10 male weanling rats each were housed in individual screen-bottom cages, maintained in an air-conditioned room at 76 to 78° F. and fed the experimental diets for 6 weeks. The basal diet, 1, consisted of 70% cereal and 30% powdered whole milk. Diets 2 and 3 contained in addition, 0.14 and 0.28% of L-lysine,[2] respectively. This is equivalent to adding 0.2 and 0.4% of L-lysine to the cereal component of the diet. These levels of lysine added to a bread diet have been shown to give marked stimulation of growth (Rosenberg et al., '52, '54). Diets 4, 5 and 6 corresponded to diets 1, 2 and 3, respectively, and contained in addition a vitamin mixture containing B vitamins, ascorbic acid and fat soluble vitamins (Sarett and Snipper, '54) to make sure that the growth response was not limited by any vitamin inadequacy. Calculations showed that all of the diets were adequate in regard to the minerals required for growth of rats. The animals were weighed each week and records were kept of food and water intake during the course of the experiment.

RESULTS

The average weight gain and food and water intake for each group of animals are shown in table 1. On diet 1, consisting of only cereal and powdered milk, the animals gained an average of 192 gm in 6 weeks, whereas on the 5 diets supplemented with L-lysine or vitamins or both, the average gains were 200 to 204 gm. There was no significant difference between the weight gain on diet 1 and that found on any of the other diets. A comparison of weight gain on diets 1 and 2 showed a non-significant p value of approximately 0.2.

[2] L-Lysine was added in the form of Darvyl (du Pont Co.) containing 95% of L-lysine · HCl and 5% of D-lysine · HCl.

Diet 4 consisted of cereal and milk supplemented with vitamins and supported an average weight gain of 202 gm. With L-lysine also added, diets 5 and 6, weight gains of 200 and 204 gm, respectively, were obtained. These data show that the growth of weanling rats on a cereal and milk diet was not significantly improved by the addition of L-lysine or a vitamin

TABLE 1

Effect of added L-lysine and vitamins on growth of male weanling rats on a diet of 70% cereal and 30% powdered whole milk

	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6
Added vitamins[1]	—	—	—	+	+	+
Added L-lysine, %	..	0.14	0.28	..	0.14	0.28
No. of rats	10	10	9	8	9	10
Wt. gain, gm per rat						
1 wk.	29	28	29	27	30	32
2 wk.	61	63	62	63	61	66
3 wk.	96	99	99	100	96	101
4 wk.	126	134	128	132	131	133
5 wk.	163	174	164	170	170	173
6 wk.[2]	192 ± 20	204 ± 20	203 ± 17	202 ± 15	200 ± 18	204 ± 38
Food intake, gm per 6 wk.[2]	501 ± 42	530 ± 30	520 ± 39	532 ± 26	527 ± 50	564 ± 89
Food efficiency, gm gain per 100 gm food[2]	38 ± 2	39 ± 3	39 ± 2	38 ± 2	38 ± 2	37 ± 6
Water intake, ml per 6 wk.	1030	1070	990	930	910	1010
ml per gm food[2]	2.1 ± 0.3	2.0 ± 0.3	1.9 ± 0.4	1.7 ± 0.2	1.7 ± 0.3	1.8 ± 0.4

[1] Sarett and Snipper ('54).

[2] Values given with standard deviations.

mixture or both. Growth on these diets was about the same as that obtained with good stock or experimental diets.

The average food intake on each of the 6 diets varied from 501 to 564 gm for the 6-week period, and the food efficiencies were calculated to be 37 to 39 gm gained per 100 gm of food. There were no significant differences in average food intake or food efficiency values among the groups on the six diets. The average water intakes were 912 to 1070 ml and ranged from 1.7 to 2.1 ml of water per gram of food. The differences between groups were not significant.

DISCUSSION

Feeding of a diet containing 70% cereal and 30% dried milk solids (a proportion containing less milk than would be used in feeding the cereal to infants) results in good growth of rats which is not significantly improved by the addition of a lysine or vitamin supplement. Milk and cereal complement each other; e.g., the low levels of riboflavin and certain amino acids in cereals are made up by the milk, and the deficiency of iron in milk is overcome by the cereal.

In the above diet about 60% of the protein was supplied by the cereal (16% protein) and 40% by the powdered milk (25% protein). This level of milk protein makes up for the amino acid deficiency in the proteins of most cereal products. This point has been emphasized by Hart ('52) in discussing the utilization of cereal proteins.

The level of lysine in the bread diet of Rosenberg et al. ('52, '54) was 0.3 to 0.4%, whereas Rose ('37) has shown that 1% of lysine is needed in the diet for good growth of rats when a complete amino acid mixture is provided. In the present study, the cereal contained 0.69% of lysine (microbiological assay) and powdered whole milk has been calculated to contain about 2% of lysine (Macy et al., '53). The lysine content of diet 1 can therefore be calculated as follows: from 70 gm of cereal, 483 mg of lysine; from 30 gm of powdered whole milk, 600 mg of lysine; total, 1083 mg of lysine/100 gm diet.

This mixture of cereal and milk contains 1.08% of lysine, which exceeds Rose's requirements for rat growth. If 39% milk solids were used in the diet (as would be obtained from a mixture of 1 oz. of cereal and 5 oz. of fluid milk), the milk alone would provide 780 mg of lysine per 100 gm diet (on a dry weight basis), and only a small amount of lysine in the cereal would be needed to bring the lysine value up to 1%.

From the data on food intake the average lysine intakes of the rats on diets 1 and 4 have been calculated for each week

TABLE 2

Change in lysine intake during growth of rats

		WEEKS AFTER WEANING					
		1	2	3	4	5	6
Diet 1	Rat weight,[1] gm	64.7	95.0	128.5	161.1	194.7	228.7
	Lysine intake, mg/day	82	111	131	137	147	164
	Lysine intake, mg/kg/day	1265	1170	1010	850	755	715
Diet 4	Rat weight,[1] gm	63.2	94.4	130.8	165.6	200.1	235.1
	Lysine intake, mg/day	81	117	140	150	162	173
	Lysine intake, mg/kg/day	1280	1245	1065	905	810	735

[1] Average midweek weights.

of the experiment. The lysine intakes per kilogram of body weight were based on the midweek weights of the rats (table 2). The data show that the lysine intake per kilogram of body weight decreased each week as the rats grew. During the first week following weaning less than 1300 mg of lysine per kilogram per day supported good growth. This is approximately half the figure of 2750 mg reported as required for growth of the 50 gm rat by Albritton ('53).

SUMMARY

The gain in weight of male weanling rats on a diet of 70% cereal and 30% powdered whole milk was not significantly

increased by the addition to the diet of 0.14 or 0.28% of L-lysine (equivalent to 0.2 or 0.4% of L-lysine added to the cereal). Addition of a supplementary vitamin mixture had no significant effect on weight gain with or without added lysine in the diet.

The diet of 70% cereal and 30% powdered milk contained approximately 1.08% lysine, which exceeds the requirement for growth, 1%, found by Rose ('37). A mixture of 61% cereal and 39% powdered milk (which is equivalent to one part cereal and 5 parts fluid milk such as used in infant feeding) contains approximately 1.2% lysine.

On the diet of 70% cereal and 30% powdered milk rats grew as well as they do on stock diets or on good experimental diets used in these laboratories. From the data on food intake during the growth period it has been calculated that the daily lysine intake decreased from about 1275 mg per kilogram of body weight during the first week after weaning (average midweek weight, 65 gm) to about 725 mg per kilogram during the 6th week (average midweek weight, 230 gm).

ACKNOWLEDGMENTS

The author wishes to thank Dr. Lawrence P. Snipper for handling some of the details of this experiment, and Wilbur Martin and John Kissel for care of the animals.

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ALKALINE PHOSPHATASE ACTIVITY AND DIET

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Biochemical Research Foundation, Newark, Delaware

(Received for publication April 6, 1956)

Significantly higher than normal values for alkaline phosphatase activity had been found for the liver of adult rats that had been maintained on a protein-deficient — high carbohydrate diet (Ross, Ely and Archer, '53; Ross and Ely, '54). The question arose whether the amount of protein included in the diet was directly correlated with the amount of hepatic alkaline phosphatase activity. Plasma and intestinal alkaline phosphatase activity was shown to be affected by the quantity (Lawrie and Yudkin, '49) or quality (Weil and Russell, '40) of lipid material in the diet. The activity of hepatic alkaline phosphatase in rats kept on specially planned rations has been found to be controlled by the absolute amount of casein and dextrose and by the proportion of casein and dextrose in the diet.

PROCEDURE

Male rats of the Wistar strain weighing 175 to 190 gm were used, 696 in all. After weaning, these rats had been maintained on a commercial food which contains approximately 23% protein.[1] The rats were then maintained for 21 to 23 days on semi-synthetic diets. These diets were designed with varying proportions of washed casein as the protein and of dextrose as the carbohydrate. The content of fat, salts and vitamins was held constant. All diets contained salt mixture USP XII, 4%; corn oil, 5%; rice bran extract,[2] 2%, supplemented with

[1] Purina Fox Food Blox.

[2] Vitab.

0.002% riboflavin. Non-nutritive fiber (NNF) was used as a filler. The rats had access to water at all times.

At the conclusion of the test feeding period, rats from each group were decapitated. Weighed pieces of liver were immediately homogenized in cold water for 30 seconds in a Waring Blendor at low speed. Sodium glycerophosphate, 5 mg/ml, at pH 9.1, was used as the substrate for the alkaline phosphatase determinations which were made at 37°C. on aliquot portions of this liver homogenate (Ross, Ely and Archer, '53). The unit of activity used was the micrograms of phosphorus liberated per milligram of fresh liver per hour.

RESULTS AND DISCUSSION

Different casein and dextrose proportions in the diet

In order to determine the effect on alkaline phosphatase activity of different casein and dextrose proportions, 9 groups of 12 rats each were maintained on 9 diets, in which the casein plus dextrose was always 80% of the total food mixture. Each diet contained 2% vitamin, 5% oil, 4% minerals and 9% non-nutritive fiber. The voluntary food intake of the rats was determined daily during the feeding period.

Alkaline phosphatase activity was found to be related (1) inversely to the amount of casein in the diet up to 30% (fig. 1); (2) beyond 40%, directly to the amount of casein in the diet; (3) directly to the amount of dextrose in the diet down to 50%; and (4) inversely to the dextrose content of the diet below 40%. Minimum enzyme values were therefore found when diets contained 30 to 40% of casein and 50 to 40% of dextrose.

The high alkaline phosphatase activity per unit weight of fresh liver found when rats were maintained on a casein-free or dextrose-free diet may represent an increase (a) in the amount of total enzyme in a given amount of tissue, (b) in amount of active enzyme, or (c) in the concentration of enzyme substance within the cell. It had been shown by Ely and Ross, ('53) that the level of hepatic alkaline phosphatase activity of

rats maintained on a protein-free diet for a considerable period of time was unchanged for the individual cell. On the other hand, the enzyme level had been shown (Ely and Ross, '54) to change more rapidly than the number of cells per unit mass of fresh tissue indicating that the increase in activity is the result of both changes in concentration and in amount.

Voluntary food intake was lowest in those rats maintained on either a casein-free or dextrose-free diet (table 1). Weight

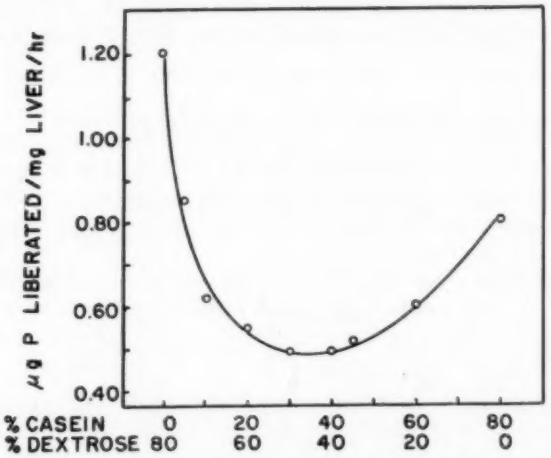

Fig. 1 Effect on hepatic alkaline phosphatase activity of diets containing different proportions of casein and dextrose.

TABLE 1

Food intake and body weight of rats maintained on diets differing in proportions of casein and dextrose

CASEIN	DEXTROSE	AVERAGE FOOD INTAKE DURING FEEDING PERIOD	AVERAGE RAT WEIGHT	
			Initial	Final
%	%	*gm*	*gm*	*gm*
0	80	11.3	186	134
10	70	15.4	186	221
20	60	14.8	185	229
30	50	17.3	185	248
45	35	15.1	186	244
60	20	12.5	186	224
80	0	12.6	186	207

loss was apparent in rats on the casein-free diet and weight gain was greatest in those receiving the 30 to 45% casein and 50 to 35% dextrose diets respectively.

Different amounts of a diet

The effect of different amounts of a diet on alkaline phosphatase activity was therefore tested. Three groups of 20 rats each were maintained on three different amounts of a diet containing 20% casein and 36% dextrose. The oil, minerals and vitamins were added to the diet in such amounts

TABLE 2

Alkaline phosphatase value and amount of food intake

	DIETARY INGREDIENTS						FOOD INTAKE	ALKALINE PHOSPHATASE ACTIVITY
	Casein	Dextrose	Oil	Minerals	Vitamins	N.N.F.		
							gm	*units*
Group I								
% of diet	20	36	5	4	2	33		0.741 ± 0.057 [1]
gm/day	3.2	5.8	0.8	0.64	0.32	5.3	16	
Group II								
% of diet	20	36	10	8	4	22		
gm/day	1.6	2.9	0.8	0.64	0.32	1.8	8	0.699 ± 0.047
Group III								
% of diet	20	36	20	16	8	0		
gm/day	0.8	1.4	0.8	0.64	0.32	0	4	0.657 ± 0.047

[1] Standard error of the mean.

that every rat consumed identical quantities of these ingredients (table 2). Rats of group I consumed on the average 16 gm of food per day. Rats of group II were given one-half this ration or 8 gm per day, and group III rats one-quarter the ration or 4 gm per day.

A slight decrease in alkaline phosphatase activity was found with decreasing food consumption; however, the activity was not found to vary significantly or appreciably with the amount of food consumed (table 2). Since the lowest activity was

found for those rats which consumed the largest amount of food and the highest activity for those which consumed the smallest amount when different proportions of casein and dextrose were consumed ad libitum, the amount of food consumed would not seem to be the determining factor for the amount of alkaline phosphatase activity.

Different amounts of casein and of dextrose

In order to determine the effect on alkaline phosphatase of different amounts of casein and of dextrose, 43 groups of 12 rats each were maintained on 43 diets in which the amount of casein was varied for each level of dextrose and vice versa. Non-nutritive fiber was added to the diets in amounts necessary to make the sum of the casein, dextrose and non-nutritive fiber 89%. Each diet contained 2% vitamins, 5% oil and 4% minerals. The data presented in figure 2 show the effect on

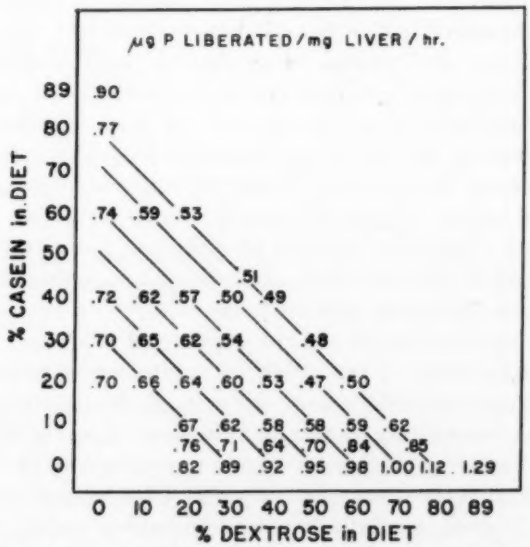

Fig. 2 Effect on alkaline phosphatase activity of diets differing in casein: dextrose ratio.

alkaline phosphatase activity of diets of the same casein content but differing in dextrose content (vertical columns), the effect of diets of the same dextrose content but differing in casein content (horizontal lines), and the effect of diets differing in both dextrose and casein content but similar in caloric content (diagonals). Standard errors have been omitted in figure 2 because the added figures would make comparisons more difficult. The differences found are statistically significant.

Variation in the level of casein or of dextrose. For casein alone, or for dextrose alone, hepatic alkaline phosphatase activity was found to rise with concentration of the constituent in the diet (fig. 2). The rise with concentration was more striking for dextrose than for casein. Addition of small amounts of casein to a dextrose diet, or of small amounts of dextrose to a casein diet, reduced the degree of the change in activity; sufficiently large amounts tended to reverse the effect so that the activity fell with increased concentration of the component in the diet. Dextrose, however, was a more potent factor than casein in producing these changes. For example, 5% casein reversed the effect for dextrose only when the dextrose concentration was 30% or less. Similarly, 10% casein reversed the effect for dextrose only at dextrose concentrations of 40% or less, while 10% dextrose reversed the effect for casein at casein concentrations as great as 60%. The lowest values for alkaline phosphatase activity therefore were found in rats fed diets containing approximately equal proportions of casein and dextrose.

Variation in the level of both casein and dextrose. A comparison was made of the effects of variation in the amounts of casein and dextrose where the sum of the casein plus dextrose was essentially constant (diagonal lines in figure 2). Since the casein used gave a nitrogen analysis of 13.8% (86% crude protein) the diets which had the same sum of casein plus dextrose were essentially constant in caloric value. Alkaline phosphatase activities for each of these groups of diets varied with the proportion of casein to dextrose. From group to

group, the activities also varied with the caloric content of the diet. The highest activity in each group of calorically similar diets was found to be directly related to the caloric content of the diet (fig. 3). On the other hand, the lowest activity in each group of these diets was found to be inversely related to the caloric content of the diet. When the caloric content was lower the amount of change was less, whether maximum or minimum.

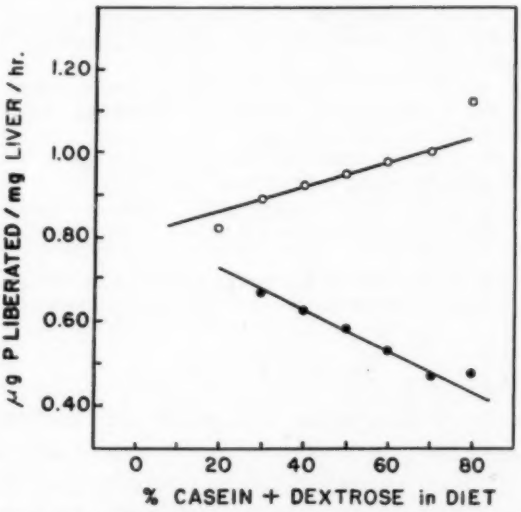

Fig. 3 Effect on alkaline phosphatase activity of groups of diets calorically similar. ○ = maximum activity in a group of calorically similar diets. ● = minimum activity in a group of calorically similar diets.

SUMMARY

Alkaline phosphatase activity of rat liver has been found to be sensitive to variation in the proportion of casein and dextrose in the diet. Activity values were found to be high not only when there was a large proportion of dextrose but also when there was a large proportion of casein in the diet. Low activity values were found when these dietary constituents were present in approximately equal proportions. Alkaline

phosphatase activity was found to be directly affected by variation in the casein content when the dextrose content was held constant and by variation in the dextrose content when the casein content was held constant. A variation in the amount or in the proportion of the dietary factors, casein and dextrose, has been shown to alter the alkaline phosphatase activity.

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PROCEEDINGS OF THE TWENTIETH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

CONVENTION HALL, ATLANTIC CITY, NEW JERSEY

APRIL 15–20, 1956

COUNCIL MEETINGS

Council meetings were held at Chalfonte-Haddon Hall, Atlantic City, on Sunday, April 15, and Monday, April 16. Formal actions of the Council are reported in the following minutes of the two business meetings held on April 17 and April 19 in Convention Hall.

SCIENTIFIC SESSIONS

One hundred and thirty-two papers were submitted by members. Ten of these were read by title, 5 were transferred to biological chemistry, one to physiology, and 12 to inter-society sessions. Thus 104 papers submitted by members, together with 18 received from other member societies (14 from biological chemistry, two from pathology, one from physiology, and one from immunology), were arranged into 12 half-day sessions. In addition, one half-day session was devoted to a symposium entitled "Amino Acids and Proteins."

BUSINESS MEETINGS

1. *Minutes.* The minutes of the 19th annual meeting, as published in the September 1955 issue of the *Journal of Nutrition,* were approved.

2. *Election.* The secretary transmitted the ballots to the Tellers' Committee, Dr. B. S. Schweigert and Dr. A. Arnold. The committee reported election results on 227 ballots cast as follows:

President: H. J. Deuel, Jr.
Vice President: R. R. Williams
Treasurer: John B. Brown (3-year term)
Councillor: E. W. McHenry
Associate Editors (4-year term beginning May 1, 1956):
Clarence P. Berg
C. G. Mackenzie
H. R. Bird

On the basis of the suggestions on the ballot returns, President Sebrell appointed the following Nominating Committee for 1956–1957:

D. V. Frost, Chairman
W. J. Darby
D. M. Hegsted
J. B. Allison
C. A. Baumann

3. *Membership.* The Secretary reported that as of April 1, 1956, there were 388 active members and 41 retired members, or a total of 429 members in the American Institute of Nutrition. The following members passed away during the year:

Dr. C. Robert Moulton, April, 1955
Dr. E. G. Ritzman, May 15, 1955
Dr. Henry C. Sherman, October 8, 1955 (Past President)
Dr. Kate Daum, December 31, 1955

The following resolution was adopted with respect to the late past president Sherman:

"The American Institute of Nutrition has learned, with regret, of the passing away on October 8, 1955, of Professor Henry Clapp Sherman, one of the prime organizers and an early president of the Institute. The members of the Institute here assembled in the twentieth annual meeting do hereby call attention to the many valuable contributions to nutritional science which were made by Professor Sherman, and to the significance of his important part in the establish-

ment, first of the *Journal of Nutrition*, and later of this Institute itself.

"The Institute wishes to pay tribute to his memory by placing this resolution in the Minutes of the Society, and by communicating these sentiments of respect and condolence to the bereaved family.

"It is especially fitting that a copy of this resolution be sent to the late Professor Sherman's daughter — Caroline Sherman Lanford — an authority on nutrition in her own right."

4. *Treasury.* The Auditing Committee, G. M. Briggs and A. E. Schaefer, submitted a written report that the report of the Treasurer was substantiated by the records of the Treasurer's Office. The Treasurer's and Auditors' reports were approved, and dues were approved at $1.00 per member for the coming year. By voice vote the outgoing Treasurer, O. L. Kline, was commended for the efficient and prompt manner in which his office functioned during his three-year term.

5. *Journal of Nutrition.* The Journal Editor, G. R. Cowgill, submitted an annual report, the summary of which follows:

Volumes of the *Journal of Nutrition*, 1955	Nos. 55, 56, 57
Number of papers submitted	188
Number of papers rejected	23
Number of papers published	150
Number of pages per article	12.3
Number of biographies	3

The papers published during the year covered research carried on in 89 laboratories representing 74 institutions. Due to the soundness and financial success of the *Journal* as reported by The Wistar Institute, its publisher, the cost of the three volumes of the *Journal* to members for the year 1956–57 has been temporarily reduced from $7.50 to $6.50. In addition, Wistar Institute has agreed to print all stationery, bills, and other printed matter required by our Institute in its normal operation at no charge.

The Editorial Board adopted in principle the policy that the *Journal* does not look with favor upon the wide use of *Federation Proceedings* abstract issue as a journal to be used in "Literature Cited." It was felt that references to abstracts in general would not be acceptable unless unusual circumstances merited their use.

The policy of publishing supplements to regular issues of the *Journal* will be continued if authors agree to the costs involved.

The Editor's report was approved with a vote of appreciation to Dr. Cowgill for the efficient handling of *Journal* affairs.

6. *Reports of Standing Committees:*

(a) Joint Committee on Nomenclature (C. K. King and E. M. Nelson). The Committee recommended that the trivial name for 1,2-dithiolane-3-valeric acid be "lipoic acid." The Committee also recommended disapproval of the recommendation of the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry that the term pyridoxine be used as a group name of naturally-occurring pyridine derivations with vitamin B_6 activity. It was felt that "vitamin B_6" is a more suitable group name. The resignation of E. M. Nelson from the Committee, effective June 30, 1956, was a part of the report.

The report was approved with a vote of thanks to Dr. Nelson for his long and faithful service on the Committee.

(b) The Committee on the Registry of Pathology of Nutritional Diseases (H. Pollack) reported that reorganization and coordination of effort with a similar committee operating under the American Society of Experimental Pathology has consumed the efforts of the group during the past year.

(c) Representative to the Food and Nutrition Board and the Division of Biology and Agriculture, N.R.C. (N. R. Ellis). The report included a recommendation that our Institute consider favorably the matter of extending financial assistance to defray the costs of distributing to high schools and junior colleges the N.R.C. publication, "The Challenge of the Life Sciences." Upon the recommendation of the Council, the report was approved and the Treasurer was authorized to pay out of the Institute Treasury a sum of $50.00 payable to Paul Weiss, Chairman, the Biology Council, N.R.C., to aid in the distribution of the above named publication.

(d) Representatives on the AAAS Council (J. H. Roe and P. B. Pearson) reported on the AAAS Council meetings held in Atlanta, Georgia, in December, 1955. The report contained the recommendations that our Institute should take advantage of additional programing of nutrition studies by extending sessions in this area at AAAS scientific meetings. The AAAS Council felt it desirable to secure opinions from affiliated societies regarding the desirability of assessing affiliated member societies modest sums ($25.00) that might be used to help defray travel expenses of delegates to Council meetings. Upon the recommendation of our representative on the AAAS Council (Dr. J. H. Roe), this proposal was not approved. The overall report was approved as presented.

(e) Representative to the Nutrition Division of F.A.O. (P. E. Howe). In the absence of Dr. Howe, Dr. J. H. Roe served as representative for our Institute at the October, 1955, meeting of the National Conference on F.A.O. Doctor Roe's report, which included the recommendation that at future National F.A.O. meetings conference on problems of nutrition education should receive consideration, was approved as presented.

7. *Nominations for Membership.* The Council received 24 nominations for membership. The following 16 were recommended by the Council and approved:

L. A. Bavetta	Olaf Mickelsen
D. E. Becker	O. Neal Miller
Louise J. Daniels	Lura Mae Odland
Burt W. Heywang	Robert Van Reen
Edward G. High	Harold M. Scott
Theodore B. Van Itallie	Martha Trulson
Herman F. Kraybill	Walter G. Unglaub
R. J. Lillie	C. M. Young

8. *Actions of the Federation Board.* The Secretary reported on the actions of the Federation Board in its Sunday meeting as follows:

(a) The proposal that an Executive Committee be set up within the membership of the Federation Board was defeated.

(b) The Public Information Committee of the Federation Board (I. M. Hoobler is American Institute of Nutrition representative on the committee) recommended the organization of a year-round Public Information service at Federation Headquarters. The proposal was approved by the Board in principle, and the committee was authorized to seek funds for the expenses involved in committee meetings that would need to be held during the coming year in order to formulate a definite plan for effecting a year-round public information service at Federation Headquarters.

(c) The proposal that in the future, abstracts of "Papers Read by Title" no longer be printed in *Federation Proceedings* was approved.

(d) The Board voted the Federation assessment of $4.00 per member, tentatively adopted in 1955, to be continued, with the acknowledgment that the $4.00 assessment is entirely used to pay costs of publishing *Federation Proceedings.*

The report was approved as presented with the recommendation that our society also consider deletion of

"Read by Title" papers from the program issue of *Federation Proceedings.*

9. *Plans for International Nutrition Congress in the United States in 1960.* C. G. King, in the absence of Chairman Paul György, reported briefly on the actions of the committee authorized last year to effect preliminary planning for the 1960 International Nutrition Congress in the United States. Pertinent action was as follows: Approval of the committee report that C. G. King serve as President of the 1960 International Nutrition delegate to the 1957 International Nutrition Congress in Paris, France. W. H. Sebrell, Jr., was designated as alternate delegate to the 1957 Congress.

The committee report was approved as presented.

10. *Miscellaneous items.*

(a) Increasing the honorarium to the Secretary's Office for secretarial assistance to $150.00 annually was approved. An honorarium of $50.00 for secretarial assistance for the Treasurer's Office was approved.

(b) The following resolution was prepared for these minutes:

RESOLUTION

Be it resolved that the American Institute of Nutrition, assembled in Atlantic City, New Jersey, in its annual meeting, April 19, 1956, place in its minutes for permanent record this statement of deep regret and sorrow at the passing of its President-Elect, Harry J. Deuel, Jr., and

Be it further resolved that high tribute be paid to President-Elect Deuel for his outstanding scientific accomplishments in research in nutritional science, for his definitive record in the preparation of scientific publications, for his extraordinary record as an ambassador in furthering international cooperation among scientists, and for his superb contribution to the fellowship of this Society, and

Be it further resolved that the Secretary of the Society be directed to send a copy of this resolution to his wife, Mrs. Grace Deuel, and to his institution, the University of Southern California.

In view of the late Dr. Deuel's many contributions in nutrition research and his devotion to American In-

stitute of Nutrition affairs, the membership adopted the recommendation of the Council that the official record will indicate the election of Dr. H. J. Deuel, Jr., as President for 1956–57 and that R. R. Williams, Vice-President Elect, be delegated to serve as Acting President for the year 1956–57.

(c) A letter commending the American Society of Biological Chemists for their Fiftieth Anniversary Celebration was forwarded by the Council.

(d) The procedure of providing an inner envelope for the ballot to be mailed inside the signed envelope, as a modified election procedure, was approved.

ANNUAL DINNER AND PRESENTATION OF AWARDS

The annual dinner of the American Institute of Nutrition was held on Wednesday, April 18, in the Madison Hotel, and was attended by 256 members and guests. The program high light was the presentation of awards by President Sebrell.

The Borden Award in Nutrition was presented to Dr. Frank M. Strong of the University of Wisconsin "for outstanding contributions on the nutritive significance of the components of milk." Doctor Strong's achievements as a research worker were described by Dr. C. A. Elvehjem. Doctor Strong responded by extending his deep appreciation to the Borden Foundation for providing the Award, to nutrition co-workers for honoring him with the Award, and to his many co-workers that have aided in his professional advancement.

The Osborne and Mendel Award was presented to Dr. A. G. Hogan, Professor Emeritus at the University of Missouri, "for his development of synthetic rations for use in nutritional studies and for his original investigations in the field of biochemistry and nutrition, which have made him one of the greatest contributors to the development of our present knowledge of animal nutrition." Doctor Hogan's achievements in nutrition were described by Dr. A. H. Smith. Doctor Hogan responded with some recollections of his early experiences with Dr. Osborne and Dr. Mendel as a graduate student.

COMMITTEES FOR 1956–57

The following are the standing committees beginning July 1, 1956:

Committee on Registry of Pathology of Nutritional Diseases

Herbert Pollack, Chairman
O. A. Bessey
W. H. Sebrell, Jr.
C. L. Pirani, Secretary
Consultant, Paul Klemperer

Representatives to the Joint Committee on Nomenclature

C. G. King
O. L. Kline

Representative to the Division of Biology and Agriculture, to the Agricultural Research Institute and to the *Food and Nutrition Board, National Research Council*

N. R. Ellis

Representatives to the American Association for the Advancement of Science

Howard A. Schneider, Section C (Chemistry)
Paul B. Pearson, Section N (Medical Science)

Representative to FAO

Paul E. Howe

Representative on Federation Public Information Committee

Icie Macy Hoobler

Respectfully submitted,

R. W. ENGEL, *Secretary*
American Institute of Nutrition

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. The following information must be submitted: Name of the Award for which candidate is proposed and as convincing a statement as possible as to the basis of the nomination (this may include a pertinent bibliography but reprints are not required). *Five copies* of all documents, including seconding statements, must be sent to the Chairman of the Nominating Committee before January 1, 1957, to be considered for the 1957 Award.

Chairman, Nominating Committee:
R. V. Boucher
Agricultural and Biological Chemistry
Pennsylvania State University
University Park, Pennsylvania

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1957 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers during the previous calendar year, but the Jury of Award may recommend that it be given for important contributions made over a more extended period of time not necessarily including the previous calendar year. The award is usually given to one person, but if in their judgment circumstances and justice so dictate, the Jury of Award may recommend that it be divided between two or more collaborators in a given research. The Jury may also recommend that the award be omitted in any given year if in its opinion the work submitted does not warrant the award. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award. Employees of the Borden Company are not eligible for this honor.

Nominations may be made by anyone. The following information must be submitted: Name of the Award for which candidate is proposed and as convincing a statement as possible as to the basis of the nomination (this may include a pertinent bibliography but reprints are not required). *Five copies* of all documents, including seconding statements, must be sent to the Chairman of the Nominating Committee before January 1, 1957, to be considered for the 1957 Award

Chairman, Nominating Committee:
C. G. MACKENZIE
Department of Biochemistry
University of Colorado School of Medicine
4200 E. 9th Avenue, Denver 7, Colorado